Medical Library

VOLUME 50 NUMBER 4

ARCHIVES

OCT 25 1932

OF

INTERNAL MEDICINE

EDITORIAL BOARD

ARTHUR BLOOMFIELD, San Francisco
N. C. GILBERT, Chicago
REGINALD FITZ, Boston
J. H. MUSSER, New Orleans
RUSSELL M. WILDER, Rochester, Minn.

OCTOBER, 1932

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH DEARBORN STREET, CHICAGO, ILLINOIS. ANNUAL SUBSCRIPTION, $5.00

Entered as Second-Class Matter, January 23, 1909, at the Postoffice at Chicago, Illinois, Under Act of Congress of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized June 14, 1918.

CONTENTS OF PREVIOUS NUMBER

SEPTEMBER, 1932. NUMBER 3

Urea Clearance Test as an Index of Renal Function. Maurice Bruger, M.D., and Herman O. Mosenthal, M.D., New York:

I. Studies of Normal Subjects.

II. The Effect of Ingestion of Carbohydrate (Dextrose).

Dietary Practices in Relation to the Incidence of Pellagra: I. A Study of Family Dietaries in Leon County, Florida. Margaret R. Sandels, Ph.D., and Eunice Grady, M.S., Tallahassee, Fla.

Undulant Fever: An Epidemic of Subclinical Infection with Brucella. Parker Dooley, M.D., Kent, Conn.

Low Voltage in the Electrocardiogram: Occurrence and Clinical Significance. Kenneth B. Turner, M.D., New York.

Treatment of Addison's Disease with Cortin (Hartman): Report of Four Cases. Perry C. Baird, Jr., M.D., and Fuller Albright, M.D., Boston.

Pericarditis. Harry L. Smith, M.D., and Fredrick A. Willius, M.D., Rochester, Minn.:

IV. Fibrinous Pericarditis and "Soldier's Patches."

V. Terminal Pericarditis.

Sedimentation Rate of Blood Corpuscles in Synovial Fluid and in Plasma: Method of Estimation and Significance in Arthritis. David H. Kling, M.D., New York.

Clinical Significance of Electrocardiograms with Large Q Waves in Lead III. Thomas Ziskin, M.D., Minneapolis.

Production of Nonfatal Vascular Sclerosis in Rabbits by Means of Viosterol (Irradiated Ergosterol). Tom Douglas Spies, M.D., Boston.

Transient Ventricular Fibrillation: The Clinical and Electrocardiographic Manifestations of the Syncopal Seizures in a Patient with Auriculoventricular Dissociation. Sidney P. Schwartz, M.D., and Abraham Jezer, M.D., New York.

Localization of Afferent Visceral Impulses in the Spinal Cord. David Davis, M.D.; Emilie U. Goode, A.B., and Soma Weiss, M.D., Boston.

Diuretic Effects and Changes in Blood and Urinary Metabolites After Digitalis in Normal and in Edematous Persons. A. B. Stockton, M.D., San Francisco.

Experimental Edema Produced by Plasma Protein Depletion. Michael J. Lepore, M.S., with the Technical Assistance of Augusta B. McCoord, Rochester, N. Y.

Book Reviews.

COPYRIGHT, 1932, BY THE AMERICAN MEDICAL ASSOCIATION

Archives of Internal Medicine

VOLUME 50 OCTOBER, 1932 NUMBER 4

INSULIN DOSAGE AND BLOOD SUGAR CHANGES

ERNEST L. SCOTT, PH.D.
AND
LOUIS B. DOTTI, M.A.
NEW YORK

Immediately following Banting's announcement of a method of preparation that would permit insulin to be obtained in sufficient quantities to make its use in experimental work practicable, and more especially with the availability of commercial preparations of insulin, there has been an avalanche of publications on carbohydrate metabolism. This was natural and to be expected. However, in spite of the multiplicity of papers, our knowledge of the deeper significance of insulin is still disappointingly poor. There seems to be, also, a certain amount of confusion concerning some of the more obvious results of insulin administration. For these reasons it seems desirable to us at this time to present an experimental review of some of the simpler aspects of insulin activity.

In view of the abundant recent and excellent reviews of the literature that have appeared there is no occasion to burden this paper with what at best could be but a repetition.[1]

One gathers from the literature that it is the general opinion that little useful knowledge may be expected from a study of the effects of the injection of small amounts of insulin. One of the reasons for this opinion is that it is thought that any change in the insulin concentration of the blood that might be brought about by such small additions would be compensated for by changes in the rate of discharge of the animal's own carbohydrate-controlling hormones. Just what hormones would be involved undoubtedly would depend on the particular circumstances of the moment. Now, perhaps, there would be an increase in the rate of the discharge of epinephrine; again, there might be a decrease in the pancreatic activity, or yet again it might be that still others of the endocrine system would be involved, i.e., the pituitary or

From the Department of Physiology, Columbia University.

1. Macleod, J. J. R., and Orr, M. D., in The Biological Standardisation of Insulin, Geneva, League of Nation's Health Organization, 1926, C. H. 398, p. 11. Macleod, J. J. R: Carbohydrate Metabolism and Insulin, London, Longmans, Green & Company, 1926. Staub, H.: Pankreas, in Bethe: Handbuch der normalen und pathologischen Physiologie mit Berücksichtigung der experimentellen Pharmakologie, Berlin, Julius Springer, 1930, vol. 16, p. 557. Cori, C. F.: Physiol. Rev. **11**:143, 1931.

the thyroid. In addition to this hypothetic difficulty, it is also thought by some that it is not possible to differentiate the small changes in the blood sugar concentration, which might be caused by such small doses, from the spontaneous variations of the initial sugar concentration.

It is the primary purpose of this paper to present a study of this particular field, that is, to develop a method, if possible, that will permit the study of the effects of small doses of insulin on the concentration of sugar in the blood, and to make use of the method, when developed, in such a study. It is the hope also that the method may be sufficiently sensitive to make possible the study of such spontaneous or physiologic changes in the concentration of blood sugar as may be presumed to be a part of the normal equilibrium of the carbohydrate metabolism. Though this is our primary purpose, it has seemed necessary or desirable to stray from it at times. Thus, for purposes of comparison, it is necessary to include a study of the effects of somewhat heavier doses. A cursory survey of the literature leaves no doubt that much has been learned from studies of the effects of the administration of large amounts of insulin, and it is highly probable that much of interest and importance still awaits investigators in this field. It is, however, hardly probable that an animal, during its entire normal life, is ever subjected to insulin changes that are at all commensurate with those imposed on it in the usual experimental procedures. It is also at least conceivable that the net results of heavy dosages are not only quantitatively but qualitatively different from those that would result from doses that might be considered to be quantitatively comparable with the changes brought about spontaneously in the ordinary course of the animal's life.

During the progress of the research there has accumulated incidentally to the main purpose a considerable body of data that may be of interest to those who desire to measure the potency of insulin preparations. It will not be out of place to consider these data from the standpoint of insulin assay.

METHODS

Rabbits were used exclusively as subjects. The animals were obtained in the open market and were selected only in regard to physical condition. No attention was paid to breed or sex.[2] When purchased, they weighed about 2 Kg. and were in early sexual maturity. After purchase, they were kept for at least two weeks on the regular regimen before they were used in experimental work. They were not subjected to experimental use oftener than once a week, with the exception that in some of the earlier work three days were allowed to elapse after the control observations and after the administration of light doses of insulin, as ¼ or 1/16 of a unit per kilogram. With heavier doses, the seven day rest interval

2. Scott, E. L.: Reducing Power (Blood Sugar) of Filtrates from Blood of Rabbits, Arch. Int. Med. **43**:393 (March) 1929.

was allowed throughout the research. The animals were kept in use so long as they remained in good condition. Some were in use for more than two years, and several attained a weight of 3.5 or 4 Kg.

In the earlier part of the work they were kept in pens with a considerable floor area, six or eight animals being kept together in a single pen. During the greater portion of the work, however, the rabbits were confined in individual cages, 12 by 15 by 20 inches. The latter system proved to be much more satisfactory since a considerably greater precision was obtained, although the results were not otherwise appreciably altered. The animals also remained in better condition and, on the average, lived longer in the individual cages than they did in the larger pens.

In that part of the earlier work in which consecutive samples were obtained the animals were fed up to the beginning of the experimental procedures, with the exception of two series, the specific purpose of which was to investigate the effects of inanition. In the remainder of the work an inanition period of about eighteen hours preceded the injections.

The research has run through a period of seven years and through a removal from one laboratory to another. As a consequence of this there have been different regimens in the diet of the animals. The diet has, however, at all times permitted what appeared to be a normal growth of immature rabbits and a full maintenance of weight in all fully grown adults. The relation of the type of diet to the effect of the insulin will be more fully discussed in a later section.

It was the custom to bring the animals to the laboratory about an hour before the beginning of the actual experimental procedures. The blood samples were obtained from the marginal ear vein after the ear was well coated with petrolatum. Hyperemia was induced by massage, or when necessary, by the application of xylene. The blood was caught in a small dish which had been dusted with powdered sodium oxalate.

The blood proteins were precipitated with phosphotungstic acid, and the sugar in the filtrate was determined by the method described by Shaffer and Hartmann.[3] The conversion table published by Duggan and Scott [4] was used for determining the sugar content of the sample.

The insulin used was the product of three different manufacturers. It was purchased in the open market in 100 unit ampules in a concentration of 20 units per cubic centimeter. The stock was kept in a refrigerator except for the time that it was in actual use in the laboratory. After being brought to room temperature, it was diluted with 0.9 per cent sodium chloride solution so that 1 cc. of the resulting mixture contained the amount to be injected per kilogram of animal weight. The volume injected, then, was always 1 cc. per kilogram, regardless of the dosage. A new dilution of insulin was made daily, unused portions being discarded. The injections were made subcutaneously in the region of the flank or the abdomen.

CONTROLS

The two major difficulties that have arisen in the study of the effects of light dosage with insulin have been mentioned. One, the question of the presence of some compensatory mechanism within the animal, is, of course, purely a problem for physiologic investigation. The other is the difficulty of differentiating those changes in the blood sugar level

3. Shaffer, P. A., and Hartmann, A. F.: J. Biol. Chem. **45**:365, 1921.

4. Duggan, W. F., and Scott, E. L.: J. Biol. Chem. **67**:287, 1926.

that occur spontaneously from those that are due to the insulin that has been administered. The latter is a problem in the precision of measurements and must be attacked from that standpoint.

There are two general methods for increasing the precision of a measurement. First, and most important, is the standardization of the material and of the technical procedures. The second method consists in multiplying the number of observations until statistical treatment is permitted.

One of us [5] has shown that in blood sugar studies on rabbits a group of fifty observations is sufficient to warrant statistical treatment and interpretation within certain determinable limits. In compliance with this, each point on every curve discussed in this paper indicates the mean of at least that number of observations, unless the contrary is specifically indicated.

The matter of the standardization of the material is somewhat more difficult, since it not only involves the original selection of the individual subjects, but also their entire subsequent care, indeed, their whole history, both before and after their entry into the laboratory. Much of this history which is pertinent cannot be known to the experimenter and undoubtedly is an important factor in the "spontaneous" variations with which all are acquainted. However, we feel that with the exercise of what care we were able to employ in the standardization of material and technic, together with the repetition of measurements, we were able to attain a precision sufficient to justify certain conclusions.

In measuring precision, we have employed the mean deviation, $\epsilon = \sqrt{\frac{\Sigma x^2}{N-1}}$, rather than the standard deviation, $\sigma = \sqrt{\frac{\Sigma x^2}{N}}$, for the reasons outlined by Scott.[5] The following derivatives of the mean deviation are also employed:

The mean deviation of the mean: $\epsilon_M = \frac{\epsilon}{\sqrt{N}}$

The mean deviation of the difference between two means: $\epsilon_{B-A} = \sqrt{\epsilon^2_B + \epsilon^2_A}$

The mean deviation of quotients: $\epsilon_{\frac{B}{A}} = \frac{\sqrt{\left(\frac{B\epsilon_A}{A}\right)^2 + \epsilon^2_B}}{A}$

Two types of controls were used. In the earliest work we followed a procedure which, for brevity, we shall refer to in further discussion as the method of consecutive sampling. In this method a blood sample was taken before the insulin was injected, and further samples were taken at stated intervals after the injection. The initial sample constitutes the control with which the later samples are to be compared. The accuracy of this form of control was checked by running a complete

5. Scott, E. L.: J. Biol. Chem. **73**:81, 1927.

series of fifty observations in which only the appropriate quantity of an isotonic solution of sodium chloride was injected. The blood samples were then taken as in a regular experiment. The results of this series indicate that, quite apart from any direct effect of the insulin, it is very probable that the concentration of sugar in the blood is influenced by the experimental procedures necessary. The method, then, is open to criticism, for, if the later samples are modified by the process of taking the earlier samples, there must be a question as to the amount of this modification in each case and of the allowance that should be made for it in estimating the true effect of the insulin. A determination of the probable extent of such modifications would constitute a respectable research in itself.[6]

"Consequently, the method of control by consecutive samples was abandoned early in the research, and the method described in one of the papers just cited [2] was substituted for it. This method, for brevity, will be referred to as the method of "independent series." In theory, it is based frankly on the principles of probability. The assumption is made that if a definite group of rabbits is cared for in a definite manner, and if the number of individuals and of observations is sufficiently large to justify statistical prediction, it may be expected that averages of observations made at different times will agree with one another within certain determinable limits. Before we ventured to make use of the method, however, it was put to experimental test and was found to hold to an unexpected precision. The details of this work were reported by Scott.[7] From the data published there it is shown that the mean blood sugar value of a group of rabbits for a given time may be predicted within determined limits. If, now, the conditions are altered by a single factor, as by the injection of insulin, and if the average concentration of sugar in the blood varies from the predicted value by more than the determined limits of precision, the difference may be attributed fairly to the effects of the insulin. This method offers the possibility of studying the effect of the insulin with a minimum number of disturbing factors. The conditions under which the control and experimental observations are made may be as nearly identical as is humanly possible, except for the fact that in the control group the animals were given injections of salt solution, while for the other group a similar volume of salt solution carried a certain amount of insulin.

Changes in the character of the material being studied, i. e., the rabbits, may be expected to engender changes in the initial level of the blood sugar concentration. Such modifications of the sugar value might very well arise through changes in the individuals subjected to study, or through changes in nutrition, temperature or other modifications in

6. Scott (footnotes *2* and 5).

7. Scott (footnote *2*, table 6).

the environmental conditions. For this reason it is necessary, when a high degree of precision is required, to accompany each experimental series of observations with a similar control series, both sets of observations being made on the same subjects.

In comparing the potency of two samples of insulin or in studying the effects of different dosages, we have frequently made up a series in the following manner:

Day of Observation	Experimental Condition
1st	First control
8th	⅛ unit, insulin sample A
15th	¼ unit, insulin sample A
22d	Second control
29th	⅛ unit, insulin sample B
36th	¼ unit, insulin sample B
43d	Final control

It will be noted that a week intervenes between any two consecutive observations. This is thought to be desirable to insure complete recovery from the effects of previous treatment. Observations are included in the final average only when the subjects studied were in a satisfactory condition throughout the period of observation. Each insulin group is adjacent to a control group and is situated between two such groups. Further, it was our intention to discard an entire series if the control groups did not agree with one another, within the limits set by statistical theory. Such a procedure was unnecessary, however, for in only one case did a lack of agreement to this degree exist, and in that one case it was necessary to discard the group because of other accidental conditions which vitiated the similarity of the component parts. It is evident from the make-up of such a series that the number of observations must be the same for each of its component parts.

THE INSULIN TIME AND DOSAGE CURVES

The purpose of the time curves is to follow the blood agar changes through a short period subsequent to the administration of several different doses of insulin. It was hoped that a study of such curves would reveal some constant characteristic that might be used in measuring the dosage of insulin.

In this work the method of consecutive sampling was made use of, for, though this method, as was pointed out in the section on "Controls," does not permit of as high a degree of accuracy as does that of independent series, the accuracy is sufficient for purposes of exploration, and this method is more rapid and less expensive than the other. In general, the curves shown in this paper are plotted in terms of percentage change in blood sugar rather than in absolute levels or in

absolute changes in level. The advantages of this form of presentation will be discussed in the section on "The Criteria of the Insulin Effect."

The results for six different doses are shown in charts 1 and 2. These charts show: (*a*) that, given a considerable number of observations, the several points may be determined with a fair degree of precision; (*b*) that the duration as well as the degree of hypoglycemia is related to the dosage; (*c*) that the curves for the lighter doses are well on the way back to the original level some time before those for the heavier doses have ceased to fall (an important point, the neglect of which occasionally gives rise to misleading statements in the literature) and (*d*) that, with the lighter doses, at least, there may be a late hyperglycemia, occurring when the returning curve overshoots the original sugar level. This reminds one of the hypoglycemia so commonly seen in dextrose tolerance curves, though, of course, it is the reverse phenomenon.

It is also instructive to modify the foregoing method of procedure so that the dextrose change for each of the time intervals studied is plotted against the several doses. This has been done in chart 3. When plotted in this manner the resulting dose curves vary in the degree of change, and in addition to this it should be noted that there is a wide variation in the form of the curves. Both of these points are of great importance in assay work, as will be brought out in the following section.

With the shortest observation period (three fourths of an hour), there results a reasonably smooth curve. At the next period (one and one-half hours), though the form of the curve is, perhaps, even more satisfactory, there has been a noticeable return toward the original (normal) value for the lighter doses, while the points for the medium doses hold their position and those for the heavier doses continue to fall. This all leads to a greater distribution of the points and so to the simulation of an increased sensitivity. This is, however, only simulation and is misleading, for it arises from the fact that the full effect of the lighter doses is missed, due to their early recovery, and consequently their effect is minimized, in relation to the others. So, though the scale is extended, the curve is distorted. After two and one-half hours the point for the lightest dose is back at the original level, so that no change is indicated, while there has been some return for each of the other doses employed. The degree of the return is, however, more marked with the medium doses than it is with the heavier ones. At three and one-half hours the point for the lightest dose is well beyond the original level, while those for the next two doses have returned almost to this level. All of the various characteristics of the several curves must be considered in

formulating any system designed for the measurement of the effect of the insulin through the determination of the concentration of the blood sugar.

THE CRITERIA OF THE INSULIN EFFECT

Before proceeding further, it is in order to select a criterion on which to base our estimation of the effect of insulin. Our primary interest lies in changes in the concentration of sugar in the blood which may be associated with changes in the concentration of insulin in the blood, whether the latter have arisen spontaneously or through the administration of insulin. That change in the blood sugar should be sought that is most intimately related to the activity of the insulin and is least affected by other factors. To be satisfactory, this change must appear with slight as well as with profound changes in the insulinemia. In administration of insulin the blood sugar changes must vary in proportion to the variations in the amount of insulin that has been administered. The curves shown in charts 1, 2 and 3 reveal several characteristics which might, a priori, be selected for this purpose.

(*a*) *The Optimum Time Interval.*—In estimating the effect of the insulin through the determination of blood sugar change, the first problem to arise is that of choosing the proper time interval between the administration of the insulin and the taking of the blood sample. In each of the curves, shown in charts 1 to 3, a very definite minimum sugar level appears. Probably the interval between the injection of the insulin and the appearance of this minimum level will occur to the investigator as the most desirable one to employ. Closer study, however, makes evident several difficulties which render this particular interval impractical. First, although the average time interval may be fairly constant in a particular group of individuals subjected to a particular set of environmental conditions, there is found to be a considerable individual variation within the group. This difficulty was early noticed by Macleod, and it is the experience of all workers in the field. It follows that, unless a number of blood samples are taken at comparatively short intervals, it is possible only occasionally to secure a sample of blood exactly on the appearance of the minimal value. Failure to secure the sample at the exact moment of the minimum must necessarily result in sugar values that are higher than the minimum. It also follows that it is not possible to determine the minimal value by the method of averages, for the minimum is a limiting value, and while chance observations may be made during the period of the lowest sugar value, the majority will either precede or follow this period. In either case, values somewhat higher than minimal values must result. On the other hand, no values below the minimal can be obtained. Any average value must, then, be in excess of the true minimal value.

Another objection to the use of the interval between the administration of the insulin and the appearance of the minimal value, which is quite as serious as the one just discussed, results from the fact that this interval varies with the insulin dose, becoming progressively longer as the dose becomes larger. It results from this that where the purpose of the observations is to determine changes in the degree of insulinemia that are related to physiologic changes or conditions, the method must be used with caution, for, as the degree of insulinemia is unknown, it follows that the time interval which should be allowed between the stimulus and the removal of the blood sample must also be unknown.

One might think, on the other hand, that where the purpose of the observations is to measure the potency of an insulin preparation, it would be possible to determine the proper interval with sufficient accuracy by means of preliminary observations. It is possible that such a procedure might be permissible, but it must be borne in mind that the interval may be expected to vary from time to time in a particular individual as well as from individual to individual. So even in this case the method would be attended with a certain inaccuracy, and the final average blood sugar must be higher than the minimum.

As has already been mentioned, it is possible to draw a series of blood samples at short intervals and to use only the one that gives the lowest sugar value. That is, it is possible to obtain data for each subject that would permit the construction of a curve for each individual such as those shown for groups in chart 1. In this manner it would be possible to obtain the actual minimal value, except for the effects of the necessary technical procedures as were discussed in the section on "Controls." In addition to these physiologic objections, the technic involved in such a procedure would be very burdensome because of the excessive number of determinations it would necessitate.

In order to mitigate these objections in some measure, it is the practice in one of the standard methods for insulin assay [1] to draw only three samples of blood at stated intervals and to use the mean value of these determinations as the criterion sought. It is true that this modification greatly reduces the number of observations; but the time curves in charts 1 and 2 show that unless the dosage is known to a fair degree of approximation very misleading conclusions may be drawn from results so obtained. This is especially true for light doses and might, in such cases, easily result in the conclusion that insulin causes a fall in blood sugar, or that it causes no change or even that it causes a rise, depending only on the dosage and the particular time intervals which happen to have been employed in the observation. It would appear, then, that in this procedure the accuracy of the final value is so far sacrificed in order to obtain better physiologic conditions

and for technical convenience that when the dosage is unknown little of practical use can be learned from it; this is certainly true for the lighter doses.

It is difficult to see how the use of the minimum blood sugar value as the criterion may be made practical, for it cannot be determined by averages, nor located by a single determination, while a series of determinations is impractical, both physiologically and technically. It is, consequently, necessary to extend the search for a practical measure of blood sugar change.

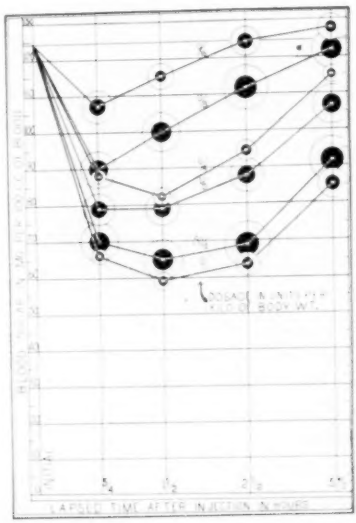


Chart 1.—Time curves for different dosages of insulin. For convenience in comparing the data, all of the values were adjusted to terms of the mean initial value of 123 mg. per hundred cubic centimeters. The circles indicate the precision with which the several points were established. The radii of the black circles correspond to ϵ_M; those of the outline circles, to $2\ \epsilon_M$. The data were obtained by the method of consecutive samples. The points on the curve for ¼ of a unit and for 1 unit were established from about one hundred and fifty observations each. The remaining points were established from fifty observations.

As was shown in the section on "Controls," the use of a single sample of blood is preferable on both physiologic and technical grounds and is permissible on the basis of its statistical significance if proper precautions are taken. The question then arises as to whether there may not be some point on the curve, other than the minimum, which could be established legitimately by averages. The practical definition of such a point must be in terms of time rather than of blood sugar level.

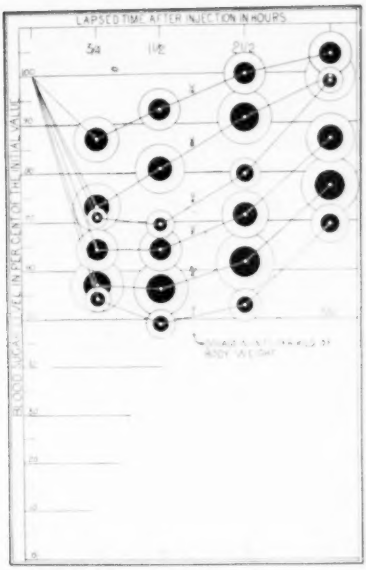


Chart 2.—Time curves for different doses of insulin. The same material as is shown in chart 1, plotted in terms of the *relative* concentration; i. e., the concentration is plotted in terms of per cent of the initial concentration, instead of in terms of the absolute values as in chart 1.

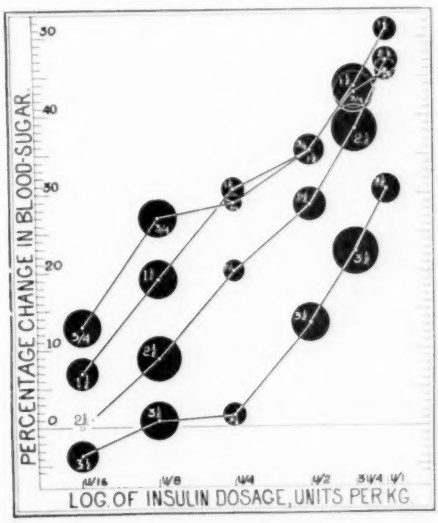


Chart 3.—Dose curves for different time intervals. This chart was plotted from the same material as is shown in charts 1 and 2. The precision is indicated by the circles, the radii of which indicate 2 ϵ_M. The figures within the circles indicate the interval, in hours, between the administration of the insulin and the drawing of the blood sample.

since any point defined in terms of blood sugar will involve many of the same difficulties that were encountered in the determination of the minimal value.

The selection of a definite interval after the administration of the insulin at which the sugar level shall be determined amounts to a determination of the rate of blood sugar change rather than a measure of the absolute change. Of course, the rate of change over any portion of the curve might, theoretically, be selected for this purpose, but an initial period has been chosen as the one which probably would be the most convenient and at the same time give the desired information with the greatest precision and accuracy.

By strict theory, the time of drawing the blood samples should be so chosen for each dose that the maximum initial velocity of change will be measured. Reference to the time curves (charts 1 and 2) will show that the time at which this maximum velocity of change occurs becomes longer as the dose is increased. From this it follows that if the maximum rate of change is to be measured the time which is allowed to elapse between the administration of the insulin and the drawing of the blood sample must be adjusted to the dose which has been administered. As the strength of the dose is unknown, since it is this that is being determined, it follows that the appropriate time interval is also unknown in any particular instance, and so this method, also, becomes impractical.

The possibility of the study of the initial rate of change for a predetermined period, which is the same for all doses, still remains. Although the rate, determined in this manner, cannot be considered as the maximum rate for all doses, the interval may be so chosen that there is a fair degree of approximation to the maximum over a considerable portion of the practical dose range.

The interval chosen should be such that the curves for the higher doses are just beginning to flatten out for the minimum level. At this time the average rate of change for the entire dose range will be somewhat less than the maximum. This arises because this flattening of the curves for the light doses occurs before those for the heavier doses have reached their maximum, so that both the lightest and the heaviest doses will show less than their maximum effect. The choice of such a period obviously is a compromise and must involve a certain amount of inaccuracy in the determination of the effect of the insulin, since very low and very high doses will both be somewhat undervalued when the interval is adjusted to give correct values for the intermediate range. However, the objections to this procedure seem to us to carry less weight than do those that may be offered against any of the other methods that we have seen proposed or that we ourselves have been able to devise.

As yet there is not a satisfactory body of data to warrant an attempt to calculate this "optimum" interval. It becomes necessary, therefore, to resort to judgment, on the basis of experience, for its choice. On this basis an interval of thirty minutes was selected as probably being very near to the optimum. Empiric results seem to have justified the choice.

(*b*) *The Most Characteristic Function of the Blood Sugar Change.*—If it is assumed that the most satisfactory procedure for sampling the blood is to draw a single specimen thirty minutes after the administration of insulin, there remains to be determined the particular function of the sugar concentration of this blood, which most precisely and accurately indicates the effect of the insulin, while it, at the same time, is relatively independent of other influences.

Two methods, which differ fundamentally in their physiologic implications, have been employed in estimating the effect of insulin on the concentration of sugar in the blood.

The method proposed by one school assumes that a definite subnormal level will be attained as the result of a definite dosage of insulin. This level, it is assumed, will be attained regardless of what the initial blood sugar level may have been. The opposed school assumes that the minimal level is in some manner related to the initial concentration. Here, again, there are two possibilities. First, it may be assumed that with a definite dose of insulin the minimal level will differ from the initial level by an amount that is determined only by the amount of insulin that has been administered and that is independent of the initial level. Second, it may be assumed that the difference between the initial and the final levels is determined, in some manner, by both the insulin dosage and the initial blood sugar concentration.

Recapitulated, the three concepts may be represented as follows:

(*a*) The final blood sugar concentration is a constant for a given dose.

(*b*) The difference between the initial and the final blood sugar concentration is a constant for a given dose.

(*c*) The ratio of the difference between the initial and final values to the initial value is a constant for a given dose.

Each of these three possibilities has been considered in the literature, but the weight of practice seems to favor the first or the second. The third possibility has, for the most part, received but scant consideration.

If the first suggestion is true, there should be a very low coefficient of correlation (Pearson's "*r*") between the initial and the final (thirty minutes after insulin) blood sugar concentrations, and a very high correlation between the initial concentration and the absolute difference between this and the final concentration due to insulin. The pre-

ceding statement would follow from the fact that the final concentration would, on this assumption, have a definite value. This value would be dependent on the insulin dosage alone and would be wholly independent of the initial value. Thus there would be no correlation between the initial and the final values, and r would necessarily be very low. Theoretically, it would be zero. On the other hand, the absolute differences between the admittedly variable initial and the assumedly constant final values must vary directly with the initial values. The consequence of this is that r must in this case approach its maximum value. Theoretically, it would be 1.

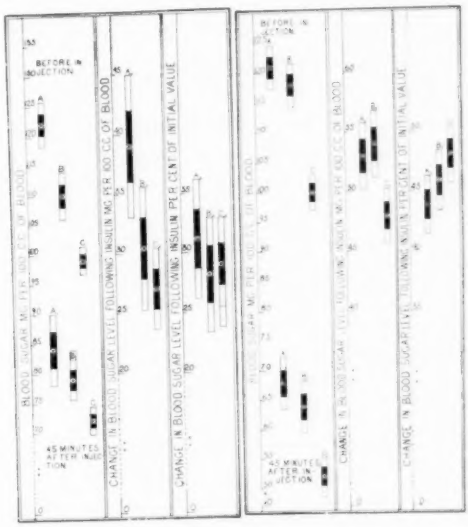
On the basis of the second assumption, a similar line of reasoning will show that there must be a high correlation between the initial and the final concentrations, while there should be a very low correlation between the initial level and the absolute difference between this and the final level. For now this difference is considered to be the definite constant value dependent only on the insulin dosage and wholly independent of the initial concentration. However, if the difference is constant, the final level must vary directly as does the initial.

If it is assumed that a given dose of insulin lowers the blood sugar concentration to a constant level or lowers it by a constant amount, then the other value must vary directly with the initial blood sugar. Further, the coefficients of correlation between these values and the initial must be very high for one and very low for the other, since the variations of the one which is dependent on the initial must have the same absolute values as do the variations of the initials themselves.

On the other hand, if it is assumed that a definite dosage of insulin will change the blood sugar concentration by a definite percentage of itself, it will result that both the final concentrations and the differences between these and the initial values will vary in the same direction as the initial amounts, but each will vary by lesser absolute amount than do the corresponding initial values. It would result from this that both the coefficients of correlation between these two values and the initial amount would take an intermediate place, since the variations of the initial values are now divided between the respective finals and differences. On the other hand, if the relative decrease depends only on the dosage of insulin, then one would expect to find a very low coefficient of correlation between the relative decreases and the initial values.

These coefficients of correlation have been examined for two groups of one hundred and fifty observations each, in which the initial blood sugar values varied over an unusually wide range. This variation of the initial values was accomplished by nutritional manipulation and, so far as we could tell, did not at any time exceed what we are pleased to term physiologic bounds.

In one of these experiments the initial blood sugar varied from 80 to 150 mg. per hundred cubic centimeters. The correlation, as represented by r, between the initial value and the value forty-five minutes after the injection of ¼ of a unit of insulin per kilogram of body weight, was 0.63; between the initial and the absolute decrease, it was 0.48, and between the initial and the relative decrease, it was only 0.11. The former two values of r are therefore intermediate, while the latter value may be considered to be so small as to be within the limits of variation and so to have no interpretable meaning.


BEFORE INJECTION
45 MINUTES AFTER INJECTION
CHANGE IN BLOOD SUGAR LEVEL FOLLOWING INSULIN MG. PER 100 CC. OF BLOOD
CHANGE IN BLOOD SUGAR LEVEL FOLLOWING INSULIN PER CENT OF INITIAL VALUE

Charts 4.—These charts were devised to illustrate the dependence of both the blood sugar concentration and the absolute drop forty-five minutes after the administration of insulin on the initial concentration, and the independence of the relative drop and the initial value. In each chart A, B and C represent the initial blood sugar levels for three different groups of observations; A_1, B_1 and C_1, absolute blood sugar level forty-five minutes after insulin; A_2, B_2 and C_2, the absolute change in level at this interval; A_3, B_3 and C_3, the corresponding percentage changes. The dosage was ¼ of a unit per kilogram of body weight for the data shown in a, and 1 unit per kilogram for those shown in b. Each point was established by fifty or more observations. The precision is indicated by the vertical bars; the black portion representing ϵ_M, and the outline portion $2\epsilon_M$.

In the other experiment the rabbits were given 1 unit of insulin per kilogram instead of ¼ of a unit, and the blood samples were taken as before, forty-five minutes after the injection. The correlation coefficients were all of the same order. The only striking difference was that

the coefficient for the correlation between the initial and the relative decrease was in this case a negligably small negative number. This change in the sign, together with the very small values of these two coefficients, makes the conclusion all but final that there is in fact no correlation at all between the relative drop and the initial.

Thus all of the coefficients of correlation are just such as would be expected on the basis of the third assumption, intermediate correlations being found for the absolute values, while no significant correlation exists between the initial and the relative change. Therefore, we may conclude that the relative change is independent of the initial blood sugar value and wholly dependent on the insulin dosage.

The results of these experiments are shown graphically in chart 4.

(*c*) *The Nature of the Proportionality Between the Blood Sugar Change and the Insulin Dosage.*—Reference to charts 5 and 6 will show that the proportionality that exists between the blood sugar changes and the dosage of insulin is not a direct one, but that over a considerable portion of its course it follows a logarithmic relationship with surprising precision. It is important to note, however, that the proportionality actually is limited in its range. No doubt one factor that underlies this loss of proportionality in the upper reaches of the curve arises from the fact, noted in paragraph *a*, that the blood samples are drawn prior to the appearance of the maximum rate of fall for these doses, while it is closely approximated in all of the lighter doses for which determinations were made. However this may be, it is possible that this is not the complete explanation, and a better understanding of the reasons for this limitation may prove to be of fundamental importance in developing a theory of carbohydrate metabolism. At present, though, we are not competent to enter on a theoretical discussion of the significance of the lack of complete proportionality, nor for the matter of that of the proportionality itself. It should be noted, though, that on the basis of the foregoing reasoning, a similar flattening of the curve is to be expected in the extreme low reaches of the curve because here it is probable that the samples have been taken after the inception of the return to the original level.

(*d*) *The Optimum Magnitude of the Insulin Dose.*—In addition to the loss of proportionality in the upper ranges, the useful portion of the curve is still further limited by the fact that the proportionality, where it exists, is logarithmic and not direct. This so affects the precision of the determinations for the heavier doses that it is not practical to differentiate the effect of one dose from that of another. At the other extreme, the precision of our best methods for the determination of blood sugar does not permit us to differentiate the sugar concentrations of blood after very small doses from those of the controls.

Chart 6 shows that the logarithmic proportionality begins to fall at a dosage somewhat less than 1 unit per kilogram. Extremely heavy dosage will hardly force a drop of more than 50 or 60 per cent of the initial value, even though a much longer interval be allowed to elapse before the blood sample is taken. Doses of $\frac{1}{16}$ of a unit per kilogram will cause a drop of 10 to 15 per cent of the initial at the end of thirty minutes. Double this dose, i. e., $\frac{1}{8}$ of a unit, will cause approximately double the relative drop, somewhere between 25 and 30 per cent; but should the dose be doubled again, that is, should $\frac{1}{4}$ of a unit be given instead of $\frac{1}{8}$, the relative drop is only about 35 per cent of the initial.

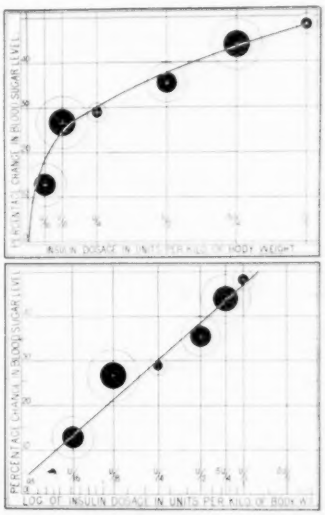

PERCENTAGE CHANGE IN BLOOD SUGAR LEVEL
INSULIN DOSAGE IN UNITS PER KILO OF BODY WEIGHT
PERCENTAGE CHANGE IN BLOOD SUGAR LEVEL
LOG. OF INSULIN DOSAGE IN UNITS PER KILO OF BODY WT.

Chart 5.—The percentage changes in the blood sugar level forty-five minutes after administration of insulin plotted against the dosage. The material is the same as was shown in chart 1. In the upper figure the relative changes are plotted against the natural values of the dosage, while in the lower figure they are plotted against the corresponding logarithms. The precision is indicated as in chart 1.

The expected effects of doses of $\frac{1}{2}$, 1 and 2 units per kilogram are, respectively, drops of 43, 50 and 53 per cent of the initial values. In other words, the relative drop for a dose of 2 units is only 23 per cent greater than that for $\frac{1}{2}$ of a unit. The corresponding changes for doses of $\frac{1}{16}$, $\frac{1}{8}$ and $\frac{1}{4}$ of a unit are respectively, 18, 27 and 35 per cent. That is, increasing the dosage by $\frac{3}{16}$ of a unit, from $\frac{1}{16}$ to $\frac{1}{4}$, will cause a greater disturbance in the blood sugar level than increasing the dosage by $1\frac{1}{2}$ units, from $\frac{1}{2}$ to 2. It would seem to be obvious,

then, that in the interest of precision the dosage should be kept as low as is consistent with an appreciable change in the blood sugar level. One eighth and ¼ of a unit per kilogram are suggested as two very satisfactory dosages with which to work. In any case, the dosage should not greatly exceed ½ of a unit per kilogram, because the loss of proportionality begins to become apparent in this region. On the other hand, it should not be much below 1/16 of a unit because of the difficulty of differentiating the small changes that might arise as a result of the insulin from the spontaneous changes which might arise from uncontrolled causes.

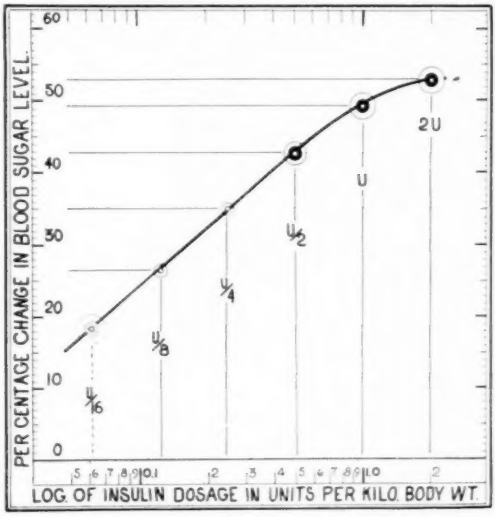


Chart 6.—The percentage drop thirty minutes after administration of insulin, plotted against the logarithm of the dose. The point for 1/16 of a unit was established from fifty observations; that for ⅛ of a unit from three hundred; for ¼ unit by four hundred, and the remaining points from one hundred observations each. The data were obtained by the use of seven samples of insulin, the product of three different manufacturers. Control was by the method of independent series. The precision is indicated as in chart 1.

NUTRITION AND THE INSULIN EFFECT

From time to time papers have appeared in which it is claimed that one or another dietary regimen influences the response of the animal to insulin. Though, in the present researches, careful attention has been given to the diet at all times, convenience or necessity has occasionally determined its modification. While every effort has been made

to keep any given dietary regimen constant through its course, there have been, in fact, several different regimens in the duration of the research. Occasionally, modifications of the mean initial sugar level have appeared which, seemingly, were associated with these changes in the diet. Associated in turn with the modifications in the initial blood sugar level, we have found the modifications in the final values and in the absolute drop due to the insulin, which would be expected from the discussion on the section on "The Criteria of the Insulin Effect." However, we never have had any evidence that the relative drop in the blood sugar level was in any manner modified by the nature, or by the

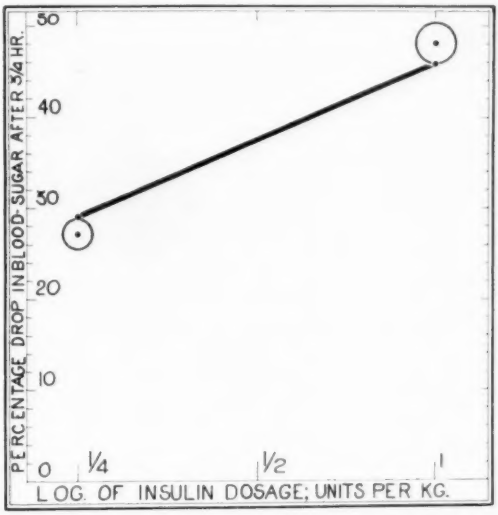


Chart 7.—A diagram to illustrate the apparent lack of influence of inanition on the effect of the insulin, when this is estimated from the relative change in blood sugar concentration. The bar represents the mean effect, as shown by one hundred and fifty observations on full fed rabbits, for each of the two points established. The dots show the effect of corresponding doses after eighteen hours of inanition. The precision with which the points on the bar are established is indicated as in chart 5. The points after inanition were established from fifty observations each. The radii of the circles indicate ϵ_M.

amount of food consumed, even though this amounted to short periods of complete inanition. A comparison of the effects of insulin after eighteen hours of starvation, with its effects on the full fed subject, is shown in chart 7.

There are two reasons, either of which might lead one to subject rabbits to a short period of inanition before using them in insulin

studies, and particularly for insulin assay. In the first place, the nutritional condition of different subjects may be presumed to be more uniform, and consequently any effects that the insulin might have may be expected to be more uniform through a group of subjects in starved animals than would be the case in animals permitted to eat as they desired. The second consideration probably results directly from this increased uniformity in the nutritional condition of the individuals of the group. It is, simply, that the initial blood sugar values of starved animals have been found to be slightly less variable than are those of well fed animals. The ideal period of inanition for insuring this increased precision appears to be from eighteen to twenty-four hours.[5] It should be noted, however, that the initial blood sugar concentration is somewhat lower under these conditions than it is in the more "normal" full fed animal. It would follow from this, in assay work, that when the assay is based on the absolute final blood sugar level, a somewhat greater precision may be obtained when food is withheld from the subjects for a short time than is possible when working with full fed animals. It should also be noted in this connection that with the lowered initial level there will be an apparent increase in the sensitivity to insulin. This is, however, only apparent and cannot be of any aid in increasing either the precision or the accuracy of the assay. It results from the fact that the absolute final level will be somewhat lower after inanition than otherwise, because of the lowered initial level. One result incidental to this is a relatively high incidence of insulin shock, as compared to that resulting from the same dosage in full fed subjects. Thus, in one series of fifty observations there were eleven cases of convulsions when the animals were full fed and twenty-six cases when the same animals were given the same insulin dosage after eighteen hours' inanition.

In any case the difference in precision is small, and when the assay is based on the relative, rather than on the absolute, change, it becomes negligible or, indeed, altogether absent. All considerations taken together, it is questionable whether the accuracy of an assay is increased by subjecting the animals to inanition in spite of the admitted increase in precision and the apparent increase in sensitivity.

THE RELATION BETWEEN THE BODY WEIGHT AND THE INSULIN EFFECT

In the Toronto method of insulin assay the attempt is made to inject a constant dose into subjects of a constant weight. Thus, when the system is adhered to strictly, the question of the relation of body weight to dosage does not arise. However, in actual practice it is frequently difficult or inconvenient to have all of the animals of precisely the same

weight, and it would be of great assistance if a system could be developed which would permit of some latitude in the weight of the animals.

The statement that the sensitivity of the animal to insulin varies inversely as the square of its body weight is attributed to Watters. Thus it is said that a 2 Kg. rabbit requires four times as much insulin to induce shock as would be required by a rabbit weighing only 1 Kg. If, however, the procedure is so changed that the dose is measured in terms of units per kilogram of body weight instead of in units per individual, it will be seen at once that if each of the aforementioned animals were given the same number of units the heavier rabbit would receive but half of the dose per kilogram that would have been given to the lighter one. Now, if it is granted that the effect of insulin is measured by the logarithm of the dose per kilogram of body weight, the underlying reason for Watters' observation is readily understood.

The relation between the weight of the rabbit and the insulin effect was studied in two series of one hundred observations each. In the first of these groups the rabbits studied varied in weight from 1.5 to 4.25 Kg. The average percentage blood sugar drop forty-five minutes after the administration of ¼ of a unit per kilogram was 30 ± 1 per cent of the initial. The coefficient of correlation (r) between the body weight and the relative drop for this group was —0.04. The second group varied in weight from 1.75 to 4.25 Kg. The percentage drop in the blood sugar concentration forty-five minutes after 1 unit of insulin per kilogram was given was 45 per cent ± 1 per cent; r was —0.01. These values of r are certainly so small as to be within their limits of error, and the conclusion is justified that when the dose is measured in units per kilogram of body weight the effects of the insulin are strictly comparable, within the limits already set, and that no further consideration of the body weight is required. Note, however, that our evidence was obtained on sexually mature rabbits. What would happen in the case of light rabbits when the small body weight is due to immaturity we are not in position to say.

It follows directly from this that, provided that they are otherwise similar, it is legitimate to include rabbits of greatly varying weight in any series of observations based on the system that we have outlined. If one considers the difference in the systems of measuring the dosage, Watters' statement is fully confirmed.

THE BEARING OF REPEATED DOSAGE ON THE INSULIN EFFECT

The question of the effect of the insulin dosage on the subsequent sensitivity of the individual to insulin is one that frequently arises. Obviously this is a matter of great importance from both the theoretical

and practical standpoints. In chart 8 the behavior of some twenty-five animals to an insulin dosage of ¼ of a unit per kilogram over a period of twenty months is shown graphically. During this period the animals were used in the ordinary course of the research and were subjected to various doses of different samples of insulin as the occasion demanded. All of the control samples for the several months studied and all of the samples one-half an hour after dosage with ¼ of a unit of insulin were assembled and the mean relative changes determined for the months which are indicated. These results were then compared with the grand mean relative change for this dosage (35 per cent ± 0.4). This comparison is shown in chart 8. In only one of the months for which averages were determined did the results differ from the standard

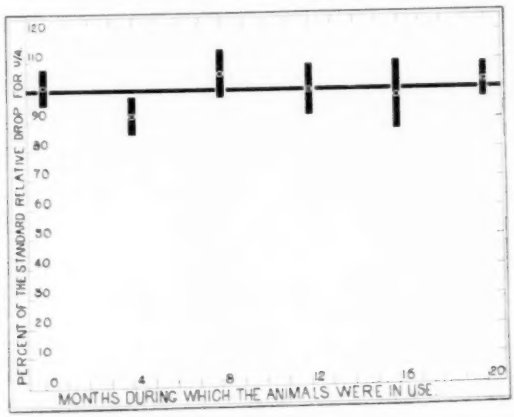


Chart 8.—A chart to illustrate the bearing of insulin dosage on the response of the animal to subsequent dosage with insulin. The data are established from a group of animals which were in use in the research for twenty or more months. The points were determined as described in the text and are plotted for every fourth month. Although during this time the animals were subjected to various dosages, the response to ¼ of a unit per kilogram is alone considered in the chart. This dose was selected for study because it was used most frequently and consequently more data were available. A study of the other doses which were used convinced us that they would have confirmed the data obtained from the ¼ unit. The points in this chart were established from fifteen to twenty observations each. See the text for the interpretation of the chart.

relative drop by more than its mean deviation, and even for this month the deviation was only slightly greater than the standard. So we find that in the only group available for this study there was rather less variation than would be expected on the basis of statistical theory. In

addition to the relatively small deviations that we find, there is a complete absence of any indication of trend, either toward a greater or a lesser sensitivity.

Our work, then, is evidence in favor of the entire independence of consecutive doses of insulin so long as they are separated by an interval of at least a week. We have no evidence as to what would happen if the insulin were administered at shorter intervals, or if given for longer periods of time.

THE BEARING OF THE CONCENTRATION OF THE INJECTED INSULIN SOLUTION ON THE RESPONSE OF THE SUBJECT

So far it has proved to be necessary to administer insulin by injection. This makes it necessary that the insulin be administered in solution, and so the question immediately arises as to the effect of the concentration of this solution on the apparent response of the subject. The effects that may arise from this cause may be either local or general. In any case they probably would be associated with absorption phenomena. It would hardly seem probable that any significant difference in behavior would result from variations in total osmotic pressure of the injected solution. The insulin is dissolved in isotonic salt solution and of itself constitutes so small a portion of the total molecular concentration, even in extreme doses, that changes in its concentration would not cause appreciable variations in the total osmotic strength.

The general problem has received theoretical attention on the part of several investigators. In fact, it would appear that its importance has been somewhat overemphasized in some cases, when one considers that, so far, there does not seem to be any satisfactory experimental evidence that, within the limits of ordinary practical necessity, there is any difference in the general effects of a given dose of insulin, whether it is administered in a concentrated or a dilute solution. It is obvious that when the dose is to be varied, either the concentration or the volume of the solution must be varied for a subject of a given weight. On theoretical grounds alone it seems quite as logical to expect variations in the effect when the volume of the injected solution is varied as it is to expect them when the concentration is changed. In the absence of direct experimental evidence which would tend to indicate that either method is unsatisfactory, it seemed to be justifiable to select the system that would prove to be the most simple in its technical use. We have, accordingly, varied the volume of the injected solution only with the weight of the subjects, 1 cc. being injected for each kilogram of body weight. The concentration, on the contrary, has been varied with the selected dose, each cubic centimeter containing the dosage designated for each kilogram of body weight.

We have been able to find no evidence in our results that this method is in any way inferior to the other or that the results obtained by the two methods do not fully agree. It is only fair, however, to state that we have designed no experiments specifically to test this point.

THE SIGNIFICANCE OF LIGHT DOSAGE

A few papers have appeared in which the use of light dosage in insulin assay is specifically attacked. The general tendency in practice seems, either tacitly or explicitly, to favor the use of fairly heavy dosage.

Since we, on the contrary, are favoring the use of comparatively light doses, it will not be out of place to discuss the significance of a few of the objections that have been raised against this practice.

Occasionally the statement has been made that light doses have no effect or that there is an "all or none behavior." It seems to us that all of our evidence is against this point of view, in whatever form it may be expressed. Against the statement that light doses have no effect we offer the evidence of our own data, as shown in charts 5 and 6, which cover the range of dosage that apparently is meant in such statements. Extrapolation would indicate that with a dosage of $\frac{1}{32}$ of a unit there should be a drop of approximately 10 per cent of the initial value. This is just outside the limits of precision of the methods that we have used for our blood sugar determinations, and so should be indicated if an appropriate series of observations were made. Such a series we do not have, but in a very short series of observations on rabbits the drop is 11 per cent of the initial. Miss Hrubetz, working in our laboratory, has unpublished data for the rat, which include a series of one hundred observations at this dosage. The relative blood sugar change for this dosage takes its proper place on a curve extrapolated from the results obtained from doses of $\frac{1}{16}$, $\frac{1}{8}$ and $\frac{1}{4}$ of a unit per kilogram. Extrapolations beyond $\frac{1}{32}$ of a unit indicate a percentage drop of such small magnitude as to be within the experimental error of the methods that are available, and so are at present incapable of experimental justification.

Moreover, on purely theoretical grounds, it is probable that extensive extrapolation is not justified. It has been shown that the proportionality of the curve falls off in its upper reaches, and that at least one reason for this lies in the fact that at the time when the samples were drawn the rate of change in the blood sugar concentration had not yet reached its maximum. While the precision of our sugar methods does not at present permit a critical study at the other end of the dose scale, the time curves shown in charts 1 and 2 would lead one to expect that the curves for extremely low doses would already be well on their way back toward the initial value at the time at which the samples were

drawn. This would lead to a flattening of the curve at this end as well, so that while direct extrapolation would indicate that a dose slightly less than $\frac{1}{128}$ of a unit per kilogram would have no effect on the blood sugar level, it is probable we would actually find that only a zero dose would have zero effect.

We are hardly justified in basing a positive theoretical discussion on such an extensive extrapolation as is necessary here, in any case, much less so when the theoretical probability is that the shape of the curve would change in the region of the extrapolation. The failure of our technic to demonstrate the extremely small changes in blood sugar level which are to be expected to result from very small doses of insulin can

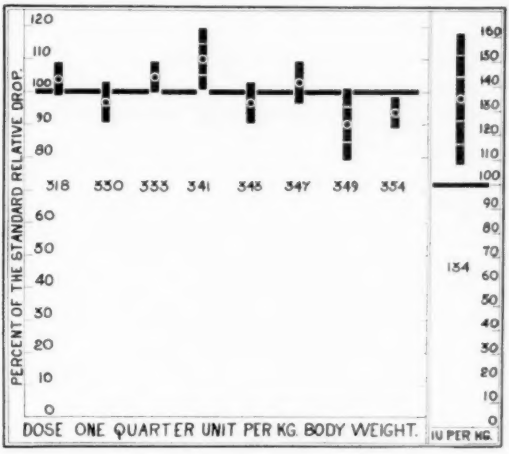


Chart 9.—Individual response to insulin. In each case the points represent the degree of response of the individual as compared to the standard response taken from chart 6. The positions of these points were located as were those of chart 8. The vertical bars measure the precision with which the points were established and represent the indicated even multiples. The points were established from twenty to thirty observations each.

hardly be interpreted as indicating that they do not exist. As insulin continues to have the expected effect as far down the curve as it can be followed technically, it is difficult to justify assumptions that it acts differently beyond this point. We are, therefore, unable to interpret our data as giving any support to the hypothesis that light doses of insulin have no effect or that insulin in any manner acts according to an "all or none" principle.

INDIVIDUALITY IN INSULIN RESPONSE

The possibility of idiosyncrasy must be borne in mind continuously in all physiologic work. Particularly in work of a quantitative nature, such as the present study, it is important to assure ourselves that our conclusions are not biased by the inclusion within the group of certain subjects that are distinctly more or less sensitive than is characteristic for the species. Unless it has been shown that such individuals do not exist, care must be exercised so to plan a research that the possible presence of variant individuals cannot bias the final results to a significant degree.

In our own work the presence of such variants was assumed, and we sought to avoid bias by using a considerable number of subjects in each of our groups, with the hope that bias would be eliminated statistically.

During the course of the work a sufficient number of similar observations were made on a few individuals to enable us to form some judgment of the sensitivity of these individuals. The results of this study are shown in chart 9. They clearly indicate not only that the animals do vary among themselves in their sensitivity to insulin, but that in an occasional animal this variation may be considerable and that it may be in either direction. So that unless the possibility of the presence of variants is considered, it may be a real menace to the reliability of results. From the fact that both hyposensitive and hypersensitive animals are shown to exist, it should be possible to eliminate bias by the use of a number of subjects.

SUMMARY

1. When the dose is measured in units per kilogram of body weight and the blood samples are drawn thirty minutes after the injection of insulin, the relative drop in blood sugar is proportional to the logarithm of the dose through a considerable portion of the practical dose range.

2. It is impractical to use doses of less than $\frac{1}{16}$ of a unit per kilogram because of the lack of sufficient precision in blood sugar methods, and of greater than $\frac{1}{2}$ of a unit per kilogram because of the loss of proportionality for doses greater than this, when the samples are taken under the conditions which we have described.

3. The dietary regimen may influence the initial blood sugar concentration and, secondarily, the absolute concentration after insulin. We were unable to demonstrate that the quantity or the nature of the food in any way affected the relative blood sugar changes after insulin.

4. There is a direct relation between the body weight and sensitivity to insulin dosage when the effect of the insulin is compared with the

logarithm of the dose, so that when the dosage is measured in terms of units per kilogram of body weight, the same results are obtained with adult rabbits of widely varying body weights as are obtained when the animals are selected to a given constant weight.

5. If insulin is not administered oftener than once a week nor for a longer period than twenty months, we have found no evidence that the response to subsequent dosage with insulin is in any way affected.

6. There is no evidence that extremely light doses do not have the full expected effect. Appearances to the contrary may as well be due to technical failure as to absence of response.

7. There is evidence that individual animals may be either hyposensitive or hypersensitive to insulin when their response is compared with the average response of a considerable group of animals.

PERNICIOUS ANEMIA

TREATMENT WITH EQUINE LIVER EXTRACT INJECTABLE EITHER SUBCUTANEOUSLY OR INTRAVENOUSLY

ARTHUR E. MEYER, PH.D.

OSCAR RICHTER, M.D.

AND

ANDREW C. IVY, M.D.

CHICAGO

In view of the obvious advantages of an injectable antipernicious anemia substance and the promising results reported by Gansslen,[1] Selander,[2] van Leeuwen,[3] Strauss, Taylor and Castle[4] and others with intravenous and intramuscular materials, this work was undertaken with the twofold objective of producing an improved injectable material that would be (*a*) of a definitely standardized potency and (*b*) of a chemical refinement sufficiently great to remove the irritating and objectionable elements and thus permit safer, more convenient and painless subcutaneous use.

In order to begin with material of highest known potency, we used equine liver prepared as reported in our previous work.[5] This method was essentially similar to that of Strauss, Taylor and Castle.[4] Absolute alcohol sufficient to bring the liquid up to 95 per cent was added to the liver extract. After standing, the precipitate was separated and dissolved in a convenient amount of water, and a phosphate buffer mixture was added in order to make the solution isotonic and to adjust the p_H of the finished product to 7.2. The potency of the extract was then standardized so that 1 cc. was equivalent to 10 Gm. of whole liver, to which 0.4 per cent of cresol was added as a preservative. The solution was then filtered and sealed in ampules.

This work was done by a grant from the Chappel Foundation for Organotherapeutic Research at Northwestern University and Cook County Hospital.

1. Gansslen, M.: Ein hockwirksamer, injizierbarer Leberextrakt, Klin. Wchnschr. **9**:2099 (Nov. 8) 1930.

2. Selander, P.: Injectable Liver Preparation, Hygiea **93**:625, 1931.

3. van Leeuwen, H. C.: Treatment of Pernicious Anemia by Intravenous Administration of Liver Extract, Nederl. tijdschr. v. geneesk. **75**:4425, 1931.

4. Strauss, M. B.; Taylor, F. H. L., and Castle, W. B.: Intramuscular Use of Liver Extract, J. A. M. A. **97**:313 (Aug. 1) 1931.

5. Richter, Oscar; Meyer, A. E., and Ivy, A. C.: The Treatment of Pernicious Anemia with Horse Liver Extract, Preliminary Report, J. A. M. A. **98**: 1623 (May 7) 1932.

Preliminary animal and laboratory tests were made to establish the safety of this material for parenteral administration: It was cultured for sterility; the absence of anaphylactic effects was shown on guinea-pigs; irritating qualities were found to be absent by subcutaneous and intramuscular injections into man, rabbits and dogs; rabbits were given injections of large doses at intervals without producing objective

TABLE 1.—*Responses of Ten Patients with Pernicious Anemia to Subcutaneous and Intravenous Injections of an Equine Liver Extract*

Case	Age	Relapse	Days on Treatment	Maximum Reticulocyte, Percentage	Changes in Hemoglobin, Percentage		Changes in Red Blood Cells, Millions		Total Dosage, Cc.	Injection Route	Comment
					Before	After	Before	After			
A.Br.	51	1	58	25.2	25	80	0.830	4.31	110.0	Subcutaneous	Patient also had a bleeding cervical polyp removed while on treatment
M.C.	60	2	48	27.6	24	83	0.840	4.17	91.0	Intravenous, 3 cc.	Total of 57 cc., continued subcutaneously, 2 cc. daily
M.J.	72	1	72	29.4	26	81	0.890	4.14	128.0	Subcutaneous	Marked psychosis; improvement
L.K.	68	3	57	5.0*	41	81	1.08	4.14	73.5	Intravenous	2.75 cc. daily
A.K.	70	2	41	36.2	21	82	0.780	4.05	82.0	Subcutaneous	Marked mental improvement
J.M.	69	1	32	22.4	33	81	1.41	4.05	64.0	Subcutaneous	Marked mental improvement
P.L.	29	2	46	12.4*	47	94	1.75	4.59	40.0	Intravenous	From 2.5 to 3.5 cc. on alternate days
A.P.	32	1	42	27.2	32	61	1.47	3.31	76.0	Subcutaneous	Still on treatment
M.G.	65	1	9	22.6	34	38	1.13	1.56	18.0	Subcutaneous	Still on treatment
N.L.	24	1	10	47.2	17	33	0.750	1.30	24.0	Subcutaneous	Entered moribund; unable to get suitable donor; still on treatment
Total			415		300	714	10.930	35.62			
Average Patient			41.5		30	71.4	1.09	3.56			
Average Net Gain					41.4		2.47				
Average Daily Gain					0.99+		59,000		1.6 cc. Average daily dose		

* Three reticulocyte responses omitted from the average were atypical owing to other treatment given a short time prior to the beginning of this treatment.

effects; the vasodepressor action was measured by blood pressure assay on dogs, the results showing that 2 cc. has a depressor action equivalent to that of 0.01 mg. of histamine, which means that 2 cc. of this material may be given subcutaneously or intravenously slowly without causing a significant histamine-like fall in blood pressure.

Through the courtesy of the attending staff of Cook County Hospital, thirteen patients with pernicious anemia were placed on treatment with this material. Daily reticulocyte counts and complete blood counts at five day intervals were made. The results are summarized in table 1.

It should be noted that in three of these cases (P. L., L. K. and A. B.) our treatment had been closely preceded by other treatment, which had absorbed the peak of the reticulocyte response.

In each of these cases several days were allowed to elapse after the prior treatment before beginning our injections, to permit the reticulocyte percentages to return to a low level. Although a secondary reticulocyte increase was observed as recorded, a typical response was not to be expected.

Omitting these three cases, the average reticulocyte increase for the group was 30.15 per cent.

The average daily gains in hemoglobin percentage and increase of erythrocytes amounted to 59,000 red blood cells and 0.99 per cent hemoglobin, which was greater than the results obtained by oral administration of the extract.[5] A complete return to the normal blood picture was induced in from six to eight weeks.

The previous history in the case of L. K. had a distinct bearing on this subject. The patient entered the hospital some months previously with 73 per cent hemoglobin and 3,200,000 red blood cells. She remained in the hospital for thirty days, during which time she was given orally large doses (equivalent to 720 Gm. daily of whole liver) of a standard council-accepted cattle liver extract, and made very slight progress, a daily gain in hemoglobin of 0.19 per cent and of red blood cells, less than 7,000. After several months she reentered the hospital, with a low blood count, 41 per cent hemoglobin and 1,080,000 red cells, and the injection treatment, as shown in table 1, was started. Her response to this treatment averaged a daily gain of 0.53 per cent hemoglobin and 53,800 red blood cells, leading to a complete remission in fifty-six days.

It is interesting to note that one patient (J. Mc.) had a chronic fibroid tuberculosis of four years' duration which was apparently arrested on entrance to the hospital. During the course of treatment, an acute process developed, which was diagnosed clinically tuberculous bronchopneumonia, and the patient had a septic temperature. In spite of the septic condition, he showed the usual reticulocyte response to parenteral liver therapy, but the red cell count has not risen above 3,200,000 in the past seven months, during which the patient has been receiving treatment.

Comparison was made of the degree of chemical refinement attained in the various liver extracts available for hypodermic injection. The solid residue per hundred grams of fresh liver represented in the various materials was found to be in: (*a*) our material, 0.405 Gm.; (*b*) product A, 1.799 Gm., and (*c*) product B, 1.05 Gm.

Thus each gram of total solids contained in our extract represents 246 Gm. of liver, compared to 55 Gm. of liver per gram of solids in product A and 95.2 Gm. of liver per gram of solids in product B.

This comparative freedom from extraneous material is probably responsible for the nonirritating properties of the extract, and permits doses from three to four times greater with correspondingly longer intervals between.

The patients received dosages varying from 2 to 3 cc. subcutaneously or intravenously, some daily and others on alternate days, the

TABLE 2.—*Responses of Patients with Atypical Pernicious Anemia with Severe Complications to Subcutaneous Injections of Equine Liver Extract*

Case	Age	Relapse	Days on Treatment	Maximum Reticulocyte, Percentage	Changes in Hemoglobin, Percentage: Before	Changes in Hemoglobin, Percentage: After	Changes in Red Blood Cells, Millions: Before	Changes in Red Blood Cells, Millions: After	Total Dosage, Cc.	Injection Route	Comment
A.B.	40	2	77	5.2*	27	81	0.870	4.06	144	Subcutaneous	Also had syphilis; antisyphilitic treatment 2 years prior to present relapse
R.Br.	59	1	26	*	57	76	2.86	3.95	48	Subcutaneous	Was on Chappel's oral liver extract prior to present therapy; had reticulocyte count 17.4 per cent; also had infected knee joint; 200 cc. of pus aspirated
J.Mc.D.	63	1	73	27.2	17	54	0.710	3.26	170	Subcutaneous	Tuberculosis and bronchopneumonia while on treatment, followed by active tuberculosis of lungs throughout treatment
Totals			176		101	211	4.44	11.27			
Average			58		33	70	1.48	3.75			
Average Net Gain					37		2.27				
Average Daily Gain					0.637		39,000				

* Three reticulocyte responses omitted from the average were atypical owing to other treatment given a short time prior to the beginning of this treatment.

average daily dosage being 1.5 cc. The subcutaneous injection of 2 or 3 cc. caused the patient no more distress than the ordinary hypodermic injections of drugs in common use. A local sensation of warmth was reported that lasted for one or two hours.

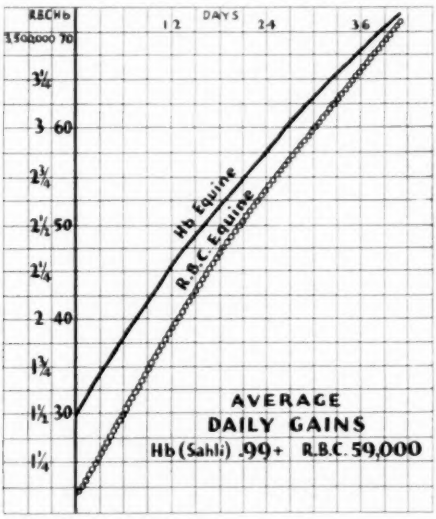
No other evidence of local or systemic reaction has been observed, objectively or subjectively with the subcutaneous treatments. This permitted routine administration by assistants or nurses.

On rapid intravenous injections into patients whose counts were below 1,000,000 red blood cells at a speed greater than 1 cc. per minute, an immediate drop in blood pressure was noticed, manifested clinically by a rapid and weak pulse. This returned to normal within a few seconds, with no further reaction. The patients reported a feel-

ing of generalized warmth and well-being a few minutes after the injections, which persisted for several hours.

In our experience with the series of patients receiving intravenous injections, no severe immediate or latent reactions were observed. A maximal and sufficient amount for each intravenous injection, we believe, should not exceed 3 cc., and injections should not be given at a rate faster than 1 cc. per minute.

The peak of the reticulocyte response was reached somewhat sooner than by oral administration, between the fourth and seventh days.



The course of progress made by ten patients with typical pernicious anemia who were given injections of equine liver extract.

Other investigators have also reported earlier responses after intramuscular or intravenous injections of liver extract.[4]

The sustained rate of gain for the red count and hemoglobin was found to be faster with the injectable material, leading to an earlier complete hematologic remission.

As previously reported, a usable injectable material has a number of advantages over oral extracts, particularly in the following cases: for patients with an aversion to liver; for those who are vomiting or are unable to assimilate orally, because of gastro-intestinal dysfunction; for stuporous or comatose patients, and for uncooperative patients, etc.

In addition, owing to the bland and harmless nature of our extract, it can be used subcutaneously as a routine treatment. Patients do not

object to this painless injection. Patients on maintenance, returning to our clinic, receive injections at intervals and are not subjected to dietary requirements.

For maintenance use, the interval between injections varies with different patients and with complications that may arise from time to time in the same patient. For this reason we have insisted that all patients return to the clinic periodically for a complete blood count to establish the individual intervals of dosage.

CONCLUSIONS

1. An injectable equine liver extract was found to be capable of producing a complete remission in cases of pernicious anemia within from six to eight weeks.

2. This extract was found to be comparatively painless on subcutaneous administration, and was safe when the precautions indicated for intravenous use were observed.

UREA CLEARANCE TEST AS AN INDEX OF RENAL FUNCTION

III. STUDIES OF PATIENTS WITH BRIGHT'S DISEASE

MAURICE BRUGER, M.D.
Oliver Rea Fellow in Medicine
AND
HERMAN O. MOSENTHAL, M.D.
NEW YORK

The material for this study is a series of ninety-four observations of the urea clearance in twenty-one cases of Bright's disease. Ten ward patients and eleven dispensary and office patients are included; the data for the latter group are of importance in the determination of the value of the urea clearance test in the ambulatory subject. The other ten patients were investigated while at complete rest in bed in the wards of the New York Post-Graduate Hospital.

Our observations covered a period of ten months. One case of acute diffuse glomerular nephritis, fifteen cases of chronic diffuse glomerular nephritis and five cases of vascular or arteriosclerotic nephritis were investigated. In two cases of chronic diffuse glomerular nephritis, while the patients were under observation, a definite so-called nephrotic component developed, as shown by increasing edema, a high amount of blood cholesterol, marked proteinuria and a low protein content and an inverted albumin-globulin ratio in the blood serum. Space does not permit the publication of a careful clinical analysis of every case investigated. Only a few will be presented in detail and the urea clearance will be correlated with the main clinical findings of all the cases in the tables.

PROCEDURE

The procedure described by Möller, McIntosh and Van Slyke,[1] as outlined in our first paper,[2] was followed throughout this investigation. Breakfast, but without coffee, was allowed each patient on the morning of the test. The ambulatory patients were sitting down during the period when the samples of urine and blood

From the Harriet Weil Memorial Fund, Department of Medicine, New York Post-Graduate Medical School and Hospital.

1. Möller, E.; McIntosh, J. F., and Van Slyke, D. D.: Studies of Urea Excretion. II. Relationship Between Urine Volume and the Rate of Urea Excretion by Normal Adults, J. Clin. Investigation **6**:427, 1928.

2. Bruger, M., and Mosenthal, H. O.: The Urea Clearance Test as an Index of Renal Function. I. Studies of Normal Subjects, Arch. Int. Med. **50**:351 (Sept.) 1932.

were collected. The ward patients were at complete rest in bed. All specimens were carefully collected and timed by one of us. The specimens of urine from patients in uremic coma were obtained by the catheter.

Urea in the blood and urine was determined by the gasometric urease method of Van Slyke.[3] The accessory chemical examination of the blood, as reported in table 1, was done according to the following methods: uric acid, Herman Brown;[4] creatinine, Folin and Wu;[5] cholesterol, Sackett;[6] total proteins, albumin and globulin, combined methods of Howe,[7] and carbon dioxide-combining power of the plasma, Van Slyke and Neill.[8] In table 1 the degree of edema and uremia and the urinary findings are expressed in plus sign grades.

RESULTS

We have had occasion to study one case of acute diffuse glomerular nephritis (table 1, case 1). One week following an acute otitis media, the patient noticed that his urine was the color of "dark coffee" and he complained of a dull aching pain in the lower part of the back. He was admitted to the ward a short time later with the diagnosis of acute diffuse glomerular nephritis. He remained in the hospital for three weeks and was discharged apparently well. The results of chemical examination of the blood and of examination of the urine were normal on several occasions before the patient's discharge. On request, he returned to the dispensary two months later stating that he felt well. Examination of the urine at that time showed albumin, occasional red blood cells and hyaline as well as finely granular casts. The urea clearance showed about 88 per cent of renal function, a figure definitely within the normal range.

Van Slyke and his associates[9] have shown that in most instances of acute diffuse glomerular nephritis, the urea clearance falls to 50 per cent or less of normal during the first two months after the onset, and that the essential for a good prognosis is that within four months after the acute onset the clearance should gradually mount to a normal level.

3. Van Slyke, D. D.: Determination of Urea by Gasometric Measurement of Carbon Dioxide Formed by the Action of Urease, J. Biol. Chem. **73**:695, 1927.

4. Brown, H.: The Determination of Uric Acid in Blood, J. Biol. Chem. **68**:123, 1926.

5. Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol. Chem. **38**:98, 1919.

6. Sackett, G. E.: Modification of Bloor's Method for Determination of Cholesterol in Whole Blood or Blood Serum, J. Biol. Chem. **64**:203, 1925.

7. Howe, in Hawk, P. B., and Bergeim, O.: Practical Physiological Chemistry, ed. 10, Philadelphia, P. Blakiston's Son & Company, 1931, p. 449.

8. Van Slyke, D. D., and Neill, J. M.: The Determination of Gases in the Blood and Other Solutions by Vacuum Extraction and Manometric Measurement, J. Biol. Chem. **61**:523, 1924.

9. Van Slyke, D. D.; Stillman, E.; Möller, E.; Ehrich, W.; McIntosh, J. F.; Leiter, L.; MacKay, E. M.; Hannon, R. R.; Moore, N. J., and Johnston, C.: Observations on the Courses of Different Types of Bright's Disease and on the Resultant Changes in Renal Anatomy, Medicine **9**:257, 1930.

TABLE 1.—*Urea Clearance Studies in Cases of Bright's Disease*

Case No.	Age	Date	Remarks	Edema	Blood Pressure	Anemia: Red Blood Cells, Millions	Anemia: Hemoglobin, per Cent	Uremia	Blood: Urea Nitrogen, Mg. per 100 Cc.	Blood: Uric Acid, Mg. per 100 Cc.	Blood: Creatinine, Mg. per 100 Cc.	Blood: Total Proteins, Gm. per 100 Cc.	Blood: Albumin, Gm. per 100 Cc.	Blood: Globulin, Gm. per 100 Cc.	Blood: CO_2 Combining Power, per Cent in Vol.	Blood: Cholesterol, Mg. per 100 Cc.	Urine: Albumin	Urine: Red Blood Cells	Urine: White Blood Cells	Urine: Casts	Urea Clearance, per Cent of Normal	Type of Renal Lesion
1 L. R. S.	19	11/12/30	Acute diffuse glomerular nephritis 3 months ago following discharging ear	Lower eyelids	108/ 68	4.1	81	0	....	....	....	....	....	....	....	....	++	++	+++	H +++ G +	..	Acute diffuse glomerular nephritis
		11/24/30	.....	0	.....	....	..	0	13.5	3.4	....	....	5.3	2.9	....	....	....	..	..	.....	..	
		12/ 3/30	Discharged from hospital	0	120/ 68	3.74	65	0	....	....	....	....	....	....	....	....	0	0	0	0	..	
		2/ 2/31	Seen in dispensary (ambulatory)	0	118/ 70	....	..	0	14.4	....	....	....	....	....	....	....	+	±	±	H + G +	88	
2 A. E. S.	23	12/19/30	Albuminuria discovered accidentally in summer of 1928 (ambulatory)	++	168/ 98	4.56	73	0	18.5	5.3	1.2	....	....	....	....	667	++++	+	+	H + G +	26	Chronic diffuse glomerular nephritis "nephrotic component"
		1/ 7/31	.....	+	150/100	....	..	0	21.7	....	....	....	....	....	....	575	++++	+	+	H + G +	40	
		2/ 9/31	Hospitalized because of increasing edema	++++	160/104	5.48	84	0	25.4	4.8	3.1	....	....	....	....	483.3	++++	+	+	H + G +	30	
		3/10/31	Ward study	+	.....	....	..	0	20.7	....	....	5.0	2.3	2.7	....	483.3	+++	+	+	H ± G +	39	
		3/20/31	Ward study	±	.....	....	..	0	22.0	....	....	5.3	2.1	3.2	....	....	++	+	+	H ± G +	29	
		3/28/31	Ambulatory	±	174/102	4.06	80	0	26.6	4.5	....	....	....	....	....	....	+++	±	+	H ± G +	30	
		5/28/31	Ambulatory	+	158/ 90	....	..	0	49.9	6.3	1.0	....	....	....	....	555.4	+++	+	+	H ± G +	23	
		9/10/31	Ambulatory	..	.....	....	..	0	32.1	3.0	1.7	....	....	....	....	553.8	+++	+	+	H ± G +	24	
3 W. G.	36	3/10/31	Acute diffuse glomerular nephritis in April, 1930	++++	146/100	3.75	72	0	74.1	....	....	....	....	....	....	250	+++	+	+	H + G +	12	Chronic diffuse glomerular

W. G. 34		merular nephritis in April, 1930															G +			diffuse glomerular
	3/20/31	Eliminating edema	++	144/100	....	..	0	46.0	....	....	....	....	....	....	....	+++++	+	+	H + G +	13	nephritis "nephrotic component"
	4/16/30	Discharged from hospital with small residual edema	+	138/ 76	3.6	72	0	28.0	....	....	5.3	2.6	1.6	....	416.6	++++	+	+	H + G +	18	secondary contracted kidney
	6/30/31	Readmitted semicomatose	++++	210/130	2.63	45	++	117.0	5.9	15.0	7.1	3.7	3.4	8.0	...	++	0	++	H ± G ++	..	uremia
	7/ 3/31	Died																			
4 48 J. O. 21	6/17/30	Albuminuria discovered accidentally 6 years ago	Eyelids	220/110	3.07	61	±	75.0	4.8	8.0	8.3	5.4	2.9	34.3	234	+++	±	++	0	...	Chronic diffuse glomerular nephritis; secondary contracted kidney with pre-uremic symptoms
	6/19/31	Signed release Aug. 8, 1931; feeling quite comfortable; blood pressure gradually fell to 150/95 but pre-uremic symptoms (nausea and headaches) continued	....	.....	....	..	±	87.1	....	....	....	....	....	....	...	++	±	++	H ± G ±	6.1	
5 17 N. E. 1	9/ 9/30	Six months ago a protracted cold; 3 months later ankles began to swell; face swollen for past 2 weeks	++	160/ 95	3.7	74	0	22.0	5.3	....	5.2	3.4	1.8	....	...	++	..	..	.....	...	Chronic diffuse glomerular nephritis; secondary contracted kidney, uremia
	11/27/30	Vomiting, headache	....	.....	2.45	43	+														
	12/ 6/30	Died Dec. 12, 1930	....	.....	....	..	++	69.8	....	....	....	....	....	....	...	....	..	..	.....	4.3	
6 42 L. G. 15	3/12/31	History of headache and vomiting for past month	±	228/122	2.38	41	+	110.3	9.6	10.5	....	....	....	....	...	++	0	+	H + G ±	...	Secondary contracted kidney, uremia
	3/20/31	Died March 31, 1931	.....	.....	....	..	++	167.0	....	....	....	....	....	....	...	....	..	..	.....	4.4	
7 38 E. D. 9	2/ 6/31	Admitted in uremic coma; indwelling catheter	+	224/160	2.65	69	++	147.0	12.2	13.4	....	....	....	....	...	+++	++	++	H 0 G +	4.5	Secondary contracted kidney, uremia
	2/ 8/31	Died																			
8 46 L. S. 18	4/ 6/31	Admitted April 5, 1931, in uremic coma	!	.....	4.1	71	++	63.0	7.2	5.7	....	....	....	....	...	+++	++	+	H ± G ±	...	Secondary contracted kidney, uremia
	4/ 7/31	.....	....	.....	....	..	++	76.3	....	....	....	....	....	....	...	+++	++	+	H ± G ±	5.6	
	4/27/31	Died May 16, 1931	±	.....	2.57	46	++	104.0	....	....	....	....	....	....	...	+++	++	+	H ± G ±	7.0	

TABLE 1.—*Urea Clearance Studies in Cases of Bright's Disease—Continued*

Case No.	Age	Date	Remarks	Edema	Blood Pressure	Anemia: Red Blood Cells, Millions	Anemia: Hemoglobin, per Cent	Uremia	Blood: Urea Nitrogen, Mg. per 100 Cc.	Blood: Uric Acid, Mg. per 100 Cc.	Blood: Creatinine, Mg. per 100 Cc.	Blood: Total Proteins, Gm. per 100 Cc.	Blood: Albumin, Gm. per 100 Cc.	Blood: Globulin, Gm. per 100 Cc.	Blood: CO_2 Combining Power, per Cent in Vol.	Blood: Cholesterol, Mg. per 100 Cc.	Urine: Albumin	Urine: Red Blood Cells	Urine: White Blood Cells	Urine: Casts	Urea Clearance, per Cent of Normal	Type of Renal Lesion
9 J. N.	15	1/12/31	Twelve years ago albumin found in urine following influenza (ambulatory)	±	144/90	4.6	90	0	19.5	....	....	....	....	....	....	....	++++	±	±	H + G +	45	Chronic diffuse glomerular nephritis
		2/16/31	.....	±	.....	....	..	0	19.7	....	....	....	....	....	....	....	++++	±	±	H + G +	42	
		4/20/31	.....	±	.....	....	..	0	24.9	....	....	....	....	....	....	....	++++	±	±	H + G +	42	
		6/25/31	Six weeks ago had cold with fever; ill in bed for 1 week	±	.....	2.9	60	0	24.4	....	....	....	....	....	....	....	++++	±	±	H + G +	26	
10 F. F.	45	2/25/31	In January, 1929, patient complained of edema of lower eyelids, vomiting and headaches (ambulatory)	±	156/104	5.04	81	0	21.4	....	....	....	....	....	....	....	±	±	±	H ± G ±	32	Chronic diffuse glomerular nephritis
11 D. G.	19	1/ 5/31	Acute diffuse glomerular nephritis following empyema in March, 1930; edema of legs first noticed in June, 1930 (ambulatory)	±	186/130	3.32	66	0	11.7	....	....	....	4.6	....	....	....	++++	+	+	H ++ G ±	35	Chronic diffuse glomerular nephritis
		2/ 6/31	.....	±	220/140	....	..	0	15.5	....	....	....	....	....	....	500	++++	+	+	H ± G ±	32	
		3/17/31	.....	±	174/112	3.3	68	0	31.8	4.9	....	....	....	....	....	379.8	++	+	+	.....	24	
		4/14/31	.....	±	.....	3.5	70	0	27.9	5.5	....	....	....	....	....	400	++++	+	+	H ++ G ++ W ++	34	
		5/26/31	.....	+	174/120	....	..	0	41.6	....	....	....	....	....	....	496	++++	+	+	H ++ G ++ W ++	39	

	5/26/31	.....	+	174/120	....	..	0	41.6	....	....	....	....	....	....	496	+	+	+	+	H ++ G ++ W ++	30	
12 25 H. R. 10	12/22/30	Edema of legs and lower eyelids first noticed in 1925 (ambulatory)	±	164/122	....	..	0	35.4	....	1.17	....	....	....	....	375	++	±	±	H ± G ±	23	Chronic diffuse glomerular nephritis
	2/12/31	.....	±	154/104	....	..	0	35.3	....	....	....	....	....	....	483.3	++	±	±	H ± G ±	23	
	4/24/31	.....	±	142/100	....	..	0	33.4	....	....	....	....	....	....	315.8	++	±	±	H ± G ±	26	
	6/11/31	.....	±	.....	....	..	0	45.5	....	....	....	....	....	....	...	++	±	±	H ± G ±	18	
13 31 E. W. 2	12/18/30	Albuminuria discovered accidentally in 1927; urine has always contained albumin and casts since then (ambulatory)	0	122/ 74	0		0	17.4	5.0	....	....	....	....	....	200	+	±	±	H + G +	101	Chronic diffuse glomerular nephritis
14 41 R. N. 7	1/17/31	Four years ago left nephrectomy for lithiasis (ambulatory)	±	140/ 85	4.84	82	±	31.7	....	1.3	....	....	....	....	...	±	±	++	H ± G ±	14	Chronic diffuse glomerular nephritis
	4/17/31	.....	±	130/ 80	....	..	±	33.8	....	....	....	....	....	....	272.2	+	±	++	H ± G ±	25	
15 47 L. S. 16	3/23/31	In March, 1929, attacks of vertigo and headaches; high blood pressure discovered then (ambulatory)	0	206/174	0		0	16.9	....	....	....	....	....	....	...	±	±	±	H ± G ±	74	Arteriosclerotic nephritis
	5/25/31	.....	0	.....	0		0	15.5	....	....	....	....	....	....	...	±	±	±	H ± G +	92	
16 55 F. M. 20	6/15/31	Diabetes for 5 years	0	186/116	0		0	27.0	....	....	....	....	....	....	...	+	±	±	H ± G ±	45	Arteriosclerotic nephritis

In a case of chronic diffuse glomerular nephritis (table 1, case 13) albuminuria and cylindruria were present continuously for the four years prior to examination with no diminution in the clearance value below the normal figures. Case 15 (table 1) illustrates the same point. It follows that there may be distinct signs indicative of a pathologic condition of the kidneys, as revealed by examination of the urine, while the urea clearance values maintain a normal level. In a large number of these cases, other tests or indexes of renal function are also normal. Recently, Wakefield, Power and Keith [10] have observed that there may be definite signs of renal disease even when all the tests of renal function that they employed are within the limits of normal variations.

TABLE 2.—*Difficulty in Obtaining Checks with the Urea Clearance in Patients with Bladder Retention*

Case No.	Age	Type of Renal Lesion	Remarks	Blood Urea Nitrogen, Mg. per 100 Cc.	Urine Urea Nitrogen, Mg. per 100 Cc.	V_c	$\sqrt{V}$	Blood Cleared of Urea, Cc. per Minute	Urea Clearance, per Cent of Normal	$\frac{U}{B}$
1 J. C. 19	58	Arteriosclerotic nephritis	.....	16.1	322.6 305.6	2.83 2.02	....	56.6 38.3	75 51	20.0 19.0
				29.2	568.8 548.5	2.21 0.66	0.81	42.8 15.0	57 28	19.4 18.8
2 M. K. 17	54	Arteriosclerotic nephritis	500 to 1,000 cc. of water by mouth necessary to obtain two hourly specimens	26.9	536.0 485.8	0.25 3.36	0.50	9.9 60.8	18 81	19.9 18.1
				22.6	440.1 393.6	0.59 2.19	0.77	15.0 38.1	28 51	19.5 17.4
3 B. F. W. 4	58	Arteriosclerotic nephritis	Voluntary control of bladder partially lost following cerebral hemorrhage three years ago	19.7	722.4 926.6	0.28 0.02	0.53 0.15	35.9 13.1	67 24	67.5 86.6

This period in the development of chronic diffuse glomerular nephritis, that is when there are albuminuria and a perfectly normal capacity of the kidney to eliminate both fluids and solids, has been called the compensated stage. Little by little, sometimes very slowly, sometimes fairly rapidly, renal function becomes progressively diminished. When this has taken place the stage of decompensation has set in. The borderline between the compensated and decompensated stage cannot be definitely defined. The gradual failure of the kidney to act with a normal degree of efficiency is the result of the formation of connective tissue and a replacement thereby of active renal parenchyma. When

10. Wakefield, E. G.; Power, M. H., and Keith, N. M.: Inorganic Sulphates in the Serum in Early Renal Insufficiency, J. A. M. A. **97**:913 (Sept. 26) 1931.

this process has advanced, as signalized by marked diminution in renal function, the so-called secondary contracted kidney presumably has developed.

Clearances consistently under 50 per cent of normal were taken as definite evidence of functional impairment of the kidney. In most of our cases of chronic diffuse glomerular nephritis (table 1, cases 2 to 14) the clearance was well below 50 per cent of normal. The urea clearance in these patients may be consistently at the same low level for months and then gradually decrease to threatening levels. In other cases, the advent of some intercurrent infection produced a sudden drop in the clearance. Case 9 (table 1) illustrates the latter point well.

Our studies of the urea clearance in Bright's disease bear out the clinical observation that patients with chronic diffuse glomerular nephritis rarely show marked improvement in the functional capacity of the kidneys irrespective of the therapeutic measures employed. This holds true when the diminution in renal function is caused by the formation of connective tissue and the obliteration of some of the renal elements. Transient occurrences that depress renal activity, notably intercurrent acute infections, as mentioned in the preceding paragraph, acute exacerbations of the nephritis or passive congestion, all of which may have a self-limited course or may be set aside by treatment, will produce temporary embarrassment of renal function, as shown by various tests, including the urea clearance. However, after such episodes have run their course, the kidney resumes its ability to functionate, and the various tests for renal function improve and assume the level at which they were before the complication occurred.

Some of our patients have maintained themselves comfortably with extremely low clearances. The patient in case 3 (table 1), studied in the wards, showed clearances ranging from 12 per cent to 18 per cent of normal and asked to be discharged because he felt fairly well. He left the hospital, but was readmitted four months later in uremic coma and died within four days. Another patient (table 1, case 4) complained of occasional vomiting and headache. There was marked retention of urea, uric acid and creatinine, and the urea clearance at that time was 6.1 per cent of normal. He signed his release from the wards two weeks later, stating that he felt much more comfortable (the blood pressure had fallen from 220 systolic and 110 diastolic to 150 systolic and 90 diastolic), but occasional vomiting and headache continued. We have not heard from the patient since.

Our observations in terminal cases of glomerular nephritis agree with those of Van Slyke and his associates.[9] When the clearance values fall to about 5 per cent of normal, uremia is impending. We have not

observed any patients with less than 5 per cent of normal clearance who lived for more than two weeks.

The elimination or deposition of large amounts of nephritic edema seems to have little effect on the urea clearance. Case 2 (table 1) was first observed as an ambulatory case, with the clinical diagnosis of chronic diffuse glomerular nephritis, added to which was a definite nephrotic component. The clearances at monthly intervals were 26, 40 and 30 per cent of normal, respectively. During this time, the edema gradually increased until it extended well up the small of the back. The patient was hospitalized for several weeks, and the edema disappeared almost completely. During his stay in the hospital, the clearances were 39 per cent and 29 per cent of normal on two separate occasions. For the three months preceding the writing of this report, he has again been studied as an ambulatory patient, the clearances practically remaining the same. Case 3 (table 1) illustrates the same point. The water balance of the sufferer with Bright's disease, therefore, often does not affect urea clearances.

Case 2 (table 1) serves to illustrate another point. In our first paper,[2] we demonstrated that moderate exercise increases the urea clearance in normal subjects. When the kidney becomes functionally impaired, however, moderate activity, such as walking, is apparently without material influence on the blood urea clearance.

In five cases of vascular or arteriosclerotic nephritis, we have been able to obtain only three satisfactory studies of clearance. We have found that the elderly patient often finds it difficult to empty the bladder completely, and that satisfactory studies of clearance cannot be made even if large amounts of water are given before and during the test. Cases 1, 2 and 3 (table 2) demonstrate the difficulty in obtaining checks for the two hours studied on the same day. Van Slyke and his collaborators,[11] in a later paper, have shown that the simple U/B ratio (when U signifies the concentration of urea in the urine and B the concentration of urea in the blood), without taking into account the volume of urine as determined for standard and maximum clearances, closely approximates the clearance values when the secretion of urine is less than 2 cc. per minute. It is often difficult, however, to gage the amount of retention and to be reasonably certain that the secretion of urine is less than 2 cc. per minute. In these instances, we have determined the U/B ratio, and although these values have been checked rather closely in some cases, we have been somewhat hesitant to include the

11. Van Slyke, D. D.; McIntosh, J. F.; Möller, E.; Hannon, R. R., and Johnston, C.: Studies of Urea Excretion. VI. Comparison of the Blood Urea Clearance with Certain Other Measures of Renal Function, J. Clin. Investigation **8**:357, 1930.

results in the general series. Case 2 (table 2) required the administration of large amounts of water before and throughout the test merely to insure the passage of two samples of urine in two hours.

We have studied a number of patients with Bright's disease (not included in the series) in whom clearance studies showed values between 55 per cent and 70 per cent of normal—figures that we consider in the doubtful range. It is probable, as Addis [12] has shown, that the occasional patient showing a somewhat lower clearance than the average normal may have no impairment of renal function. As the clearance falls, the odds that any given patient has no impairment of renal function are, of course, increased. In such cases, it is advisable, and indeed necessary, to consider all the clinical data as well as other tests for renal function before a definite conclusion is reached.

COMMENT

Addis [12] has maintained that the elimination of urea by the kidney is proportional to the blood urea content and the amount of renal secreting tissue. He assumed, therefore, that any factor expressing the relationship between the output of urea and the urea content of the blood (under standard conditions of urine excretion) is indicative of the amount of secreting tissue in the kidney. The urea clearance test of Möller, McIntosh and Van Slyke [1] is an expression of this relationship. The problem of renal function, however, is not the problem of urea excretion alone. We would rather believe that the activity of the kidney is composed of different functions, which to a certain degree act independently of one another.

The elimination of urea is an important excretory function of the kidney, and it is often the first to become affected in Bright's disease. In these instances, urea clearance tests may imply some impairment of renal function, while other functional tests prove to be negative. Derangement of kidney function in the early stages of Bright's disease, however, is not always demonstrated by excretion of urea. We shall be able to show later that the occasional case may present itself with a definite degree of retention of uric acid in the blood (in the absence of a gouty diathesis) or with a diminished ability of the kidney to concentrate the urine, in the presence of normal clearance values. Recently, Wakefield, Power and Keith [10] have observed cases of Bright's disease with retention of inorganic sulphates in the serum and normal clearance values. Obviously, the complexity of normal renal activity is reflected in its functional pathology. It seems imperative, therefore, especially in the early stages of Bright's disease, to determine the efficiency of the

12. Addis, T.: Renal Function and the Amount of Functioning Tissue, Arch. Int. Med. **30**:378 (Sept.) 1922.

kidneys from all possible angles before any conclusions are reached as to the presence or absence of functional impairment.

The urea-excreting ability of the kidney, expressed as the urea clearance, nevertheless, is a valuable clinical adjunct in the study of renal function. In many instances, when observed at regular intervals over a period of time, clearance values indicate the clinical progress of the case and may be of definite prognostic significance.

One can infer from the data obtained in a large series of normal subjects,[13] that patients with Bright's disease showing clearances above 75 per cent of normal probably have no impairment of renal function. This statement is made with a certain degree of reservation, since we have stressed the fact that this is not true in all cases. Values between 75 per cent and 50 per cent of normal should be considered in the doubtful range, because in view of some of the results obtained in normal subjects, it is evident that the urea clearance may be considerably lower than the average normal value with no decrease in renal efficiency. Values below 50 per cent always imply impairment of renal function. We agree with Van Slyke, Stillman and their co-workers [9] that in the chronic nephritides, clearance figures below 20 per cent may be considered as indicating the terminal stage of Bright's disease, although in some such cases months have elapsed before uremia has developed. Pre-uremic symptoms, such as nausea, vomiting and headache, are usually present when the clearance has fallen to 10 per cent of normal, but with values less than 5 per cent all the uremic manifestations are usually evident, and death occurs within a few days.

The urea clearance is in some instances little affected by the elimination or deposition of nephritic edema. This is of distinct advantage in any test of renal function. It is of equal value whether studied in the ambulatory or in the recumbent patient, provided there is definite renal insufficiency. With clearance values below 50 per cent of normal, moderate exercise, such as walking, apparently does not influence the amount of blood cleared of urea in a unit period of time.

In marked prostatic obstruction or incontinence from any cause, when it is impossible to gage even approximately the output of urine, clearance studies or the calculation of the U/B ratio cannot be satisfactorily made, unless catheterization is resorted to. The occasional contact with such cases and the accuracy required in collecting and timing the specimens of urine and in the determination of urea in the blood and urine detract somewhat from the use of this ratio as a routine measure in an active hospital service or in a physician's office.

13. Möller, McIntosh and Van Slyke (footnote 1). Bruger and Mosenthal (footnote 2). Goldring, W.: Studies of the Kidney in Acute Infection. II. Observations with the Urea Clearance Test in Acute Rheumatic Infection, J. Clin Investigation **10**:345, 1931.

SUMMARY

Ninety-four observations of the urea clearance in twenty-one patients with Bright's disease are reported. The urea clearance test is shown to be a valuable clinical adjunct in the study of renal function. Clearance values above 75 per cent of normal usually, though not always, suggest no impairment of renal function. Values between 75 per cent and 50 per cent should be considered in the doubtful range, and other functional tests should be carried out before any conclusions are drawn as regards the status of renal function. Clearance figures below 50 per cent of normal always imply decreased renal efficiency. In cases of Bright's disease with definite impairment of renal function, moderate exercise is without any influence on the urea clearance. The elimination or deposition of nephritic edema is shown in some instances to have little effect on the blood urea clearance.

UREA CLEARANCE TEST AS AN INDEX OF RENAL FUNCTION

IV. THE UREA CLEARANCE TEST IN RELATION TO OTHER TESTS AND MEASURES OF RENAL FUNCTION

MAURICE BRUGER, M.D.
Oliver Rea Fellow in Medicine
AND
HERMAN O. MOSENTHAL, M.D.
NEW YORK

The urea clearance studies in twenty-one cases of Bright's disease, which formed the nucleus of the preceding paper in this series,[1] will be correlated in the following pages with various other tests and measures of renal function. Van Slyke, McIntosh, Möller, Hannon and Johnston[2] and Johnston[3] have compared the urea clearance with the concentrations of urea, uric acid, creatinine and hemoglobin in the blood, the excretion rate of phenolsulphonphthalein and the urea concentration ratio in nephritic subjects. Our work permits us to compare our findings concerning the blood urea clearance in subjects with Bright's disease with the concentrations of urea, uric acid and creatinine in the blood, the blood count (hemoglobin and red blood cell count), the two hour test for renal function and the urea concentration ratio $\frac{\text{urine urea concentration}}{\text{blood urea concentration}}$

METHODS

Urea in blood and urine was determined by the gasometric urease method of Van Slyke,[4] uric acid by the method of Herman Brown[5] and creatinine by the

From the Harriet Weil Memorial Fund, Department of Medicine, New York Post-Graduate Medical School and Hospital.

1. Bruger, Maurice, and Mosenthal, H. O.: The Urea Clearance Test as an Index of Renal Function: III. Studies of Patients with Bright's Disease, Arch. Int. Med., this issue, p. 544.

2. Van Slyke, D. D.; McIntosh, J. F.; Möller, E.; Hannon, R. R., and Johnston, C.: Studies of Urea Excretion: VI. Comparison of the Blood Urea Clearance with Certain Other Measures of Renal Function, J. Clin. Investigation **8**:357, 1930.

3. Johnston, C.: The Relationship of Blood Uric Acid Content to the State of Renal Function in Nephritis, J. Clin. Investigation **9**:555, 1931.

4. Van Slyke, D. D.: Determination of Urea by Gasometric Measurement of the Carbon Dioxide Formed by the Action of Urease, J. Biol. Chem. **73**:695, 1927.

5. Brown, H.: The Determination of Uric Acid in the Blood, J. Biol. Chem. **68**:123, 1926.

method of Folin and Wu;[6] hemoglobin readings were obtained with the Dare instrument; the red cell count was done in the usual way, and the two hour renal function test was carried out according to the procedure of Mosenthal.[7]

The table mentions all the cases studied; however, only a few of the tests carried out are reported in each instance; the results that are not charted confirmed those that are offered, and a presentation of them would lead to much unnecessary duplication. The constituents of the blood and the various tests for renal function studied cover all the ranges from normal values to figures obtained with maximal impairment of renal function.

THE UREA NITROGEN CONTENT OF THE BLOOD

MacKay and MacKay[8] and Möller, McIntosh and Van Slyke[9] have shown that more than half of the functioning tissue of the kidneys may be destroyed before the blood urea nitrogen rises above normal limits. The maximum normal blood urea nitrogen used as a basis for their conclusions was 23 mg. per hundred cubic centimeters, obtained by MacKay and MacKay[10] in a study of 278 observations on 161 normal persons (presumably on their usual diet).

Mosenthal[11] and Addis and Watanabe[12] had previously shown that in normal persons the blood urea content varies more or less directly with the protein intake, and these observations were further supported by MacKay and MacKay.[10] It appears incorrect to make comparisons between functional tests of the kidney and the blood urea content in patients with Bright's disease whose protein intake is usually restricted when the maximum normal urea content of the blood is taken from a series of normal subjects on their usual diet. From clinical observations

6. Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol. Chem. **38**:98, 1919.

7. (*a*) Mosenthal, H. O.: Renal Function as Measured by the Elimination of Fluids, Salt, Nitrogen and the Specific Gravity of the Urine, Arch. Int. Med. **16**: 733 (Nov.) 1915; (*b*) Renal Function as Measured by the Elimination of Fluids, Salt, Nitrogen and the Specific Gravity of the Urine: II. The Effect of High, Low and Normal Diets, Arch. Int. Med. **22**:770 (Dec.) 1918.

8. MacKay, E. M., and MacKay, L. L.: The Relation Between the Blood Urea Concentration and the Amount of Functioning Renal Tissue, J. Clin. Investigation **4**:127, 1927.

9. Möller, E.; McIntosh, J. F., and Van Slyke, D. D.: Studies of Urea Excretion: IV. Relationship Between Urine Volume and Rate of Urea Excretion by Patients with Bright's Disease, J. Clin. Investigation **6**:485, 1928.

10. MacKay, E. M., and MacKay, L. L.: The Concentration of Urea in the Blood of Normal Individuals, J. Clin. Investigation **4**:295, 1927.

11. Mosenthal, H. O.: Metabolism in Nephritis, in Barker, L. F.; Hoskins, R. G., and Mosenthal, H. O.: Endocrinology and Metabolism, New York, D. Appleton and Company, 1922, vol. 4, p. 311.

12. Addis, T., and Watanabe, C. K.: The Causes of Variation in the Concentration of Urea in the Blood of Young Healthy Adults, Arch. Int. Med. **19**:507 (April) 1917.

in all types of cases, it seems to us that a blood urea nitrogen of more than 15 mg. per hundred cubic centimeters on a protein intake of about 40 Gm. per day suggests retention of urea. We have found (data to be published) that the average city dweller, left to his own dietetic initiative, consumes about 40 Gm. of protein per day.

Whatever value of urea nitrogen in the blood is taken as an indication of retention of urea, there is no doubt that marked degrees of renal insufficiency may exist, as measured by the urea clearance test, while decidedly normal values for urea nitrogen are found in the blood. Case 11*a* (table) reveals a urea nitrogen of 11.7 mg. per hundred cubic centimeters of blood with only 35 per cent of the average normal clearance. Our findings, in general, agree with those of MacKay and MacKay,[8] Möller, McIntosh and Van Slyke[9] and Van Slyke, McIntosh, Möller, Hannon and Johnston,[2] that normal values for urea nitrogen may be obtained in the blood of patients with Bright's disease who have lost as much as 60 per cent of their renal function, as measured by the urea clearance test. Inasmuch as we look on a urea nitrogen of more than 15 mg. per hundred cubic centimeters of blood as suggestive evidence of urea retention in the blood of patients on a restricted protein intake, we cannot state definitely, as Van Slyke and his collaborators [2] have done, that the urea nitrogen of the blood does not become elevated until the clearance has fallen to 50 per cent of the average normal value. We have observed cases suggesting slight retention of urea in the blood with clearances somewhat above 50 per cent of normal.

THE URIC ACID CONTENT OF THE BLOOD

In the majority of cases of Bright's disease, the uric acid is the first substance in the blood to become augmented with increased impairment of renal function. Chace and Myers,[13] Myers, Fine and Lough [14] and Baumann, Hansmann, Davis and Stevens [15] have shown that an increase in the uric acid content of the blood may be one of the earliest signs of renal insufficiency. The blood uric acid, however, is also increased in gout, leukemia, pneumonia, essential hypertension, pernicious anemia and several other conditions. Excluding such clinical entities in any case of Bright's disease, the uric acid content of the blood is significant. Uric acid, like urea, is largely of exogenous origin, and in any uncom-

13. Chace, A. F., and Myers, V. C.: The Value of Recent Laboratory Tests in the Diagnosis and Treatment of Nephritis, J. A. M. A. **67**:929 (Sept. 23) 1916.

14. Myers, V. C.; Fine, M. S., and Lough, W. G.: The Significance of the Uric Acid, Urea, and Creatinine of the Blood in Nephritis, Arch. Int. Med. **17**:570 (April) 1916.

15. Baumann, L.; Hansmann, G. H.; Davis, A. C., and Stevens, F. A.: The Uric Acid Content of the Blood Compared with the Renal Dietary Test, Arch. Int. Med. **24**:70 (July) 1919.

plicated case of Bright's disease with a restricted protein intake, a uric acid content of 5 or 6 mg. per hundred cubic centimeters of blood is certainly indicative of renal insufficiency.

Recently, Johnston [3] compared the uric acid content of the blood with the urea clearance in thirty cases of renal disease. He concluded that the blood uric acid is of little value as an indicator of renal function, and he found that as much as 80 per cent of the urea excretory power of the kidney may be lost and normal values for uric acid still be found in the blood. Our results agree with those of Johnston [3] in this respect, namely, that moderately increased blood uric acid (from 4 to 6 mg. per hundred cubic centimeters of blood) may accompany either slight or maximal renal damage, but we have found in the large majority of cases with renal insufficiency, as measured by the blood urea clearance test, a definite increase in the uric acid content of the blood above the normal maximum of 4 mg. per hundred cubic centimeters (table, cases 2*b*, 4, 10 and 11*b*).

We have also made the observation that the uric acid content of the blood may be slightly increased in cases of Bright's disease with no impairment of renal function as measured by the urea clearance. Case 13 (table) is an example. Our belief is that the uric acid may be increased in the blood as one of the earliest signs of impairment of renal function. There are many who do not agree to this conception, because the uric acid may rise because of extrarenal influences, and its interpretation is difficult on this account. However, if the history and physical findings in any case point to involvement of the kidneys, an elevation of the blood uric acid often indicates an incipient diminution of the functional excretory activity of the kidneys. In many instances the two hour test corroborates this, since nocturnal polyuria and a slight lowering of the urinary specific gravity may accompany the increment in the uric acid of the blood. This question admits of various interpretations, but as we have followed the clinical progress of patients, we have concluded that an elevation of the uric acid in the blood in many instances is indicative of a diminution in the excretory power of the kidneys, even though other tests for renal function may show little or no impairment.

THE CREATININE CONTENT OF THE BLOOD

There has been some doubt as to what is really measured by the various methods in use for determining creatinine in the blood. Behre and Benedict [16] are of the opinion that creatinine is present in the blood in extremely small quantities, and that what is actually measured is some

16. Behre, J. A., and Benedict, S. R.: Studies in Creatine and Creatinine Metabolism, J. Biol. Chem. **52**:11, 1922.

Case Number	Type of Renal Lesion	Blood: Urea Nitrogen, Mg. per 100 Cc.	Blood: Uric Acid, Mg. per 100 Cc.	Blood: Creatinine, Mg. per 100 Cc.	Blood Count: Hemoglobin, per Cent	Blood Count: Red Blood Cells, Millions	Two Hour Test	Specific Gravity (Last Two Figures)	Volume Night Urine, Cc.	U/B: With Urine Volume Less Than 1 Cc. per Minute	U/B: With Urine Volume Between 1-2 Cc. per Minute	U/B: With Urine Volume More Than 2 Cc. per Minute	Volume of Blood Cleared of Urea: With Urine Volume Less Than 2 Cc. per Minute, Cc.	Volume of Blood Cleared of Urea: With Urine Volume More Than 2 Cc. per Minute, Cc.	Blood Urea Clearance per Cent of Normal Cs=54 Cm=75
1	Acute diffuse glomerular nephritis...	14.4	....	....	..	....	Day.....	05-18		61.7	....	....	50.0	....	92.4
							Night...	12	700	53.4	....	....	45.3	....	83.9
2A	Chronic diffuse glomerular nephritis	18.5	5.3	1.2	75	4.36	Day.....	12-19		22.6	....	....	12.4	....	22.9
							Night...	11	510	24.7	....	....	16.0	....	29.6
2B	Chronic diffuse glomerular nephritis	25.4	4.8	3.1	..	....	Day.....	09-16		20.1	....	....	16.3	....	30.2
							Night...	12	560	20.6	....	....	15.9	....	29.5
2C	Chronic diffuse glomerular nephritis	26.6	4.5	....	80	4.06	Day.....	07-15		19.2	....	....	15.8	....	29.3
							Night...	09	610	19.9	....	....	16.3	....	30.2
3	Chronic diffuse glomerular nephritis	74.1	10.2	....	72	3.75	Day.....	12-16		....	6.2	....	6.7	....	12.5
							Night...	14	320	....	5.5	....	6.7	....	12.4
4	Chronic diffuse glomerular nephritis	87.1	4.8	8	61	3.07	Day.....	07-10		....	3.2	....	3.4	....	6.3
							Night...	10	150	3.8	....	....	3.3	....	6.0
5	Chronic diffuse glomerular nephritis	69.8	....	....	30	1.75	Day.....	10-12		5.5	....	....	2.3	....	4.2
							Night...	09	500	5.5	....	....	2.4	....	4.5
6	Chronic diffuse glomerular nephritis	166.6	7.4	14.3	40	2.17	Day.....	08-12		....	2.4	....	2.4	....	4.5
							Night...	12	700	2.5	....	....	2.4	....	4.4
7	Chronic diffuse glomerular nephritis	147.3	12.2	13.4	69	3.65	Day.....	08-11		3.4	....	....	3.0	....	5.6
							Night...	11	48	2.7	....	....	1.9	....	3.5
8	Chronic diffuse glomerular nephritis	104	6.2	13	46	2.57	Day.....	10-10		....	3.7	....	3.9	....	7.2
							Night...	10	250	....	3.8	....	3.8	....	7.1

9	Chronic diffuse glomerular nephritis	24.4	....	....	60	2.9	Day.....			20.6	....	....	13.4	....	24.9
							Night...			19.9	....	....	14.2	....	26.3
10	Chronic diffuse glomerular nephritis	21.4	5	....	81	5.05	Day.....	09–14		....	....	10.9	....	29.6	34.5
							Night...	06	1000	....	11.8	....	16.3	....	30.1
11A	Chronic diffuse glomerular nephritis	11.7	....	....	66	3.32	Day.....	06–11		....	18.3	....	19.2	....	35.5
							Night...	06	600	....	16.9	....	19.1	....	35.4
11B	Chronic diffuse glomerular nephritis	31.8	5	....	68	3.3	Day.....	02–06		....	10.8	....	13.1	....	24.2
							Night...	04	860	....	9.7	....	13.2	....	24.3
12	Chronic diffuse glomerular nephritis	35.3	....	1.2	.	....	Day.....	05–12		....	9.6	....	12.7	....	23.5
							Night...	06	1000	....	10.1	....	12.5	....	23.2
13	Chronic diffuse glomerular nephritis	17.4	5	....	90	4.76	Day.....	17–30		59.5	....	....	57.7	....	106.7
							Night...	10	1200	52.2	....	....	51.2	....	94.7
14	Chronic diffuse glomerular nephritis	31.7	....	1.3	82	4.84	Day.....	02–06		....	....	4.3	....	10.1	13.4
							Night...	01	410	....	....	3.0	....	10.8	14.4
15	Chronic diffuse glomerular nephritis	14.6	2.5	....	96	6.4	Day.....	04–18		....	....	16.8	....	56.1	74.7
							Night...	10	590	....	....	12.1	....	45.4	60.4
16	Chronic diffuse glomerular nephritis	16.2	3.7	....	91	4.74	Day.....	18–28		....	34	....	37.3	....	69.0
							Night...	06	510	....	26.5	....	33.7	....	62.3
17A	Arteriosclerotic Bright's disease.....	16.9	5	....	84	5.02	Day.....	11–27		55.8	....	....	36.0	....	66.6
							Night...	23	510	50.7	....	....	43.8	....	81.0
17B	Arteriosclerotic Bright's disease.....	15.5	4.8	1.7	..	....	Day.....	13–18		....	....	30.7	....	69.2	92.0
							Night...	16	720	....	....	25.8	....	71.3	94.9
18	Arteriosclerotic Bright's disease.....	26.9	....	....	94	4.78	Day.....	11–16		25.8	....	....	24.9	....	46.0
							Night...	08	1100	24.9	....	....	23.3	....	43.1
19	Arteriosclerotic Bright's disease.....	16.1	....	....	72	3.9	Day.....	08–12		....	....	20.0	....	56.6	75.3
							Night...	10	1100	....	....	19.0	....	38.3	51.0
20	Arteriosclerotic Bright's disease.....	26.9	....	....	..	....	Day.....	11–15		19.9	....	....	9.9	....	18.3
							Night...	14	320	....	....	18.1	....	60.8	80.8
21	Arteriosclerotic Bright's disease.....	10.7	3.4	....	90	5.77	Day.....	09–14		67.5	....	....	35.9	....	69.5
							Night...	18	500	86.6	....	....	13.1	....	24.2

unknown chromogenic substance. This, of course, does not detract from any clinical application of the so-called creatinine determinations, since in marked renal insufficiency this chromogenic substance is retained in the blood.

It has been appreciated for a long time that creatinine rises in the blood only when renal function becomes markedly impaired (Chace and Myers,[13] Myers, Fine and Lough[14] and Myers and Lough[17]). Our results show that normal creatinine values may be found in the blood with as much as 85 per cent of kidney function lost, as measured by the urea clearance test (table, cases 2*a*, 12 and 14). When the urea clearance has fallen to about 5 per cent of normal and uremia is impending, the creatinine begins to mount in the blood (table, cases 4, 6, 7 and 8). This is in full accord with the findings of Van Slyke and his associates.[2]

THE HEMOGLOBIN PERCENTAGE AND THE RED BLOOD CELL COUNT

The fact that anemia is associated with impairment of renal function has been appreciated since the days of Bright. More recently, Brown and Roth[18] showed that the degree of anemia is directly proportional to the increase of creatinine in the blood, and they stressed the prognostic value of their data. Ashe[19] has shown, in a detailed study of 136 cases of Bright's disease, that the severity of the anemia and the degree of renal insufficiency run a parallel course. The anemia at times resembles an extreme secondary type and at others, the pernicious type with a high color index. He observed the latter state more frequently in patients with marked renal insufficiency, and expressed the belief that the damage to the liver usually present in such cases may account for the relatively high hemoglobin percentage.

Van Slyke and his associates[2] have shown that the hemoglobin content of the blood is likely to fall much later in the disease than the blood urea clearance. This seems to be in accord with the view held by Ashe,[20] who believes that renal insufficiency must exist for a variable period

17. Myers, V. C., and Lough, W. G.: The Creatinine of the Blood in Nephritis: Its Diagnostic Value, Arch. Int. Med. **16**:536 (Oct.) 1915.

18. Brown, G. E., and Roth, G. M.: The Anemia of Chronic Nephritis, Arch. Int. Med. **30**:817 (Dec.) 1922. The Prognostic Value of Anemia in Nephritis, J. A. M. A. **81**:1948 (Dec. 8) 1923.

19. Ashe, B.: The Hemoglobin Percentage and the Red Blood Cell Count in Bright's Disease, Myocardial Insufficiency and Hypertension, Arch. Int. Med. **44**: 506 (Oct.) 1929.

20. Ashe, B.: Anemia in Bright's Disease, in Mosenthal, H. O.: Diagnosis and Treatment of Variations in Blood Pressure and Nephritis, Oxford Monographs on Diagnosis and Treatment, vol. 7, edited by H. O. Christian, New York, Oxford University Press, 1930.

before the anemia becomes manifest. The uremic patients with less than 10 per cent of renal function as measured by the blood urea clearance who showed hemoglobin contents above 80 per cent of normal, as reported by Van Slyke, McIntosh and their co-workers,[2] were patients with acute diffuse glomerular nephritis in whom the marked degree of renal insufficiency was transient.

In patients with chronic diffuse glomerular nephritis with urea clearances definitely below the normal level in whom renal insufficiency has existed for several weeks or months, secondary anemia is usually present, the intensity of which generally, though not invariably, is in direct proportion to the degree of impairment of renal function and the duration of the insufficiency.

The retention uremia associated with chronic diffuse glomerular nephritis is, in our experience, always accompanied by a marked degree of anemia. Cases 4 to 8 (table) illustrate the severe degrees of anemia (and the high color indexes) that may be encountered in the uremia terminating the chronic nephritides. This is, in reality, a restatement of the fact that the anemia is as a rule directly proportional to the degree of renal insufficiency.

THE RATIO OF URINE UREA CONCENTRATION TO BLOOD UREA CONCENTRATION

Van Slyke and his associates [2] compared the standard blood urea clearance and the ratio of urine urea concentration to blood urea concentration (U/B ratio) in a series of nephritic subjects and concluded that of all the tests they examined, the determination of the U/B ratio was the only one that approximated in sensitiveness the measurement of the blood urea clearance. Harrison [21] stressed the fact that consistent results may be obtained with the U/B ratio in nephritic subjects if the volume of urine is less than 2.5 cc. per minute. Van Slyke and his collaborators [2] have shown that the U/B ratio approximates the standard clearance only when the urine volume is less than 2 cc. per minute.

We have tabulated our results in three columns (table)—cases with volumes of urine of less than 1 cc. per minute, those with volumes between 1 and 2 cc. per minute and those with volumes of more than 2 cc. per minute. A study of our protocols shows that, with urine volumes of more than 2 cc. per minute, the U/B ratio bears no relationship to clearance values. A close approximation between the U/B ratio and the standard clearance is obtained with urine volumes between 1 and 2 cc. per minute. With urine volumes much less than 1 cc. per minute (oliguria) the U/B ratio deviates appreciably from the standard clearance.

21. Harrison, G. A.: On Urea Tests of Renal Function, Brit. J. Exper. Path. 3:28, 1922.

THE TWO HOUR TEST FOR RENAL FUNCTION

The two hour test for renal function was formulated by Hedinger and Schlayer in 1914. It was adapted by Mosenthal,[7a] in 1915, to diets customary in the United States. Subsequently, in 1918, it was shown that types of diet within extremely wide limits were suitable for the test (Mosenthal [7b]). The various concentration and dilution tests, so successively initiated by Volhard, are essentially similar to the two hour test for renal function, since they depend on the interpretation of the volume of urine eliminated and on the power of the kidney to concentrate and dilute the urine, as measured by the specific gravity. For more than ten years, we have carried out the two hour test on patients who were ambulatory and eating their customary diet, with satisfactory results. The table shows the degree of variation in the specific gravity of the urine excreted during the day and the specific gravity and the volume of the urine excreted during the night.

In the interpretation of the results of the two hour test, all possible modifying factors have been excluded; that is, influences other than renal, such as cardiac decompensation, the elimination of edema, pyelitis, cystitis, etc., which distort the true readings of specific gravity and volume, have been eliminated. Normal renal function is demonstrated by a 9 point variation or more of the specific gravity of the urine voided in twenty-four hours. The maximal specific gravity of any one specimen should be 1.020 or higher. The night volume of urine (measured from three hours after the evening meal to the rising hour the following morning) should not exceed 725 cc. Mosenthal [22] stated:

> With diminished renal function, the night volume is prone to increase so that it may be necessary for the patient to void one to four or more times a night. The nocturnal polyuria apparently is a process of elimination which compensates for a diminished excretion during the day. Nocturnal polyuria often is a very early sign of impairment of renal function in Bright's disease; it is of great significance when present.

The standard urea clearance of Möller, McIntosh and Van Slyke [23] expresses the number of cubic centimeters of blood of which the urea content is concentrated into 1 cc. A normal standard clearance of 54 cc. of blood (100 per cent of urea clearance) indicates that the kidneys concentrate the blood urea fifty-four times under these conditions. It follows, therefore, that the standard clearance may be interpreted as the concentrating power of the kidney, and the corollary presents

22. Mosenthal, H. O.: Variations in Blood Pressure and Nephritis, Oxford Monographs on Diagnosis and Treatment, vol. 7, edited by H. O. Christian, New York, Oxford University Press, 1930.

23. Möller, E.; McIntosh, J. F., and Van Slyke, D. D.: Studies of Urea Excretion. II. Relationship Between Urine Volume and the Rate of Urea Excretion by Normal Adults, J. Clin. Investigation **6**:427, 1928.

itself that the standard urea clearance and the specific gravity of the urine (which is really an index of the concentration of urinary solids by the kidney) should parallel one another. Our results, however, do not show this to be true in all cases.

Cases 2*a*, 2*b* and 2*c* (table) show that there may be fairly good concentration of the day urine with only 30 per cent of renal function as measured by the urea clearance test. In such instances, however, the specific gravity of the night urine shows that some renal insufficiency exists. Although specific gravity readings do not actually parallel the urea clearance values, all cases with urea clearances of less than 50 per cent of the average normal value suggest impairment of renal function as measured by the two hour test.

With clearance values below 10 per cent of normal (table, cases 4 to 8) there is always fixation of the specific gravity of the urine at a low level (from 1.010 to 1.012), indicative of maximal renal insufficiency. With clearance figures between 10 per cent and 30 per cent of normal, the variation of the specific gravity of the day urine is diminished; the maximal specific gravity of 1.020 is never reached; the specific gravity of the night urine is always low, and there may be nocturnal polyuria (table, cases 2, 3, 11*b*, 12 and 14). With urea clearance values between 30 per cent and 50 per cent of normal, the two hour test usually shows definite impairment of renal function in one or more of the characteristics mentioned (table, cases 10, 11*a* and 18).

In occasional instances, the urea clearance test showed graver impairment of renal function than was suggested by the result of the two hour test. In other cases, normal urea clearance values were obtained in the presence of a definitely decreased specific gravity of the night urine and marked nocturnal polyuria. In the latter case (table, case 13), the interpretation of the two hour test was slight renal insufficiency, whereas the clearance test gave normal figures.

COMMENT

A careful study of all the data obtained by the various measures and tests for renal function in the study of Bright's disease yields several pertinent facts. It is our belief that no single test for the determination of renal efficiency is to be preferred above all others in the evaluation of renal function, and this is especially true when little or no impairment exists.

The urea clearance test is a valuable index of renal function and in the great majority of cases shows a decreased ability of the kidney to excrete urea before there is any evidence of nitrogen retention in the blood. It should be emphasized, however, that occasionally a patient

may show a definite increase of uric acid in the blood which could be explained only on the basis of slight renal insufficiency, while the urea clearance test shows normal renal function. We have observed cases with marked nocturnal polyuria explainable only on the same basis with normal blood urea clearance values. Wakefield, Power and Keith [24] demonstrated identical findings for the inorganic sulphates in the serum in early renal insufficiency. In the critical analysis of cases of Bright's disease with only slight impairment of renal function, it is necessary, indeed imperative, to carry out as many of the tests and measures of renal function as possible in order to determine the status of renal efficiency from all angles. No single test evaluates renal function in all its physiologic and pathologic aspects.

The degree of anemia in Bright's disease parallels the amount of renal insufficiency, provided the impairment has existed for some time. It follows, therefore, that the urea clearance test will show impairment of renal function for some time before the anemia becomes apparent. In the chronic nephritides, in which a definite degree of renal insufficiency has existed for several weeks and longer, the degree of anemia is indicative of the amount of functional impairment as determined by the urea clearance test.

When satisfactory clearance studies cannot be made because of bladder retention, incontinence, etc., Van Slyke and his associates [2] suggested calculating the U/B ratio, when the urinary rate is known to be less than 2 cc. per minute. Under such conditions, they state, the U/B ratio approximately equals the standard clearance. Our results agree with those of Van Slyke and his collaborators, but it should be pointed out that in oliguric patients the U/B ratio may deviate appreciably from the standard clearance and the calculation of the percentage of normal renal function may give somewhat higher readings. In general, it may be stated that the U/B ratio may be used in place of the standard clearance when for some reason accurate urine volumes cannot be obtained and the volume is known to be less than 2 cc. per minute. In patients with bladder retention or incontinence, it appears to us more advisable to obtain definitely timed specimens of urine by catheterization and thus determine either the standard or the maximum urea clearance. We have always felt hesitant about accepting the U/B ratio as an index of renal function when it was impossible to gage even approximately the urine output.

Inasmuch as the blood urea clearance under standard conditions of urinary excretion determines the ability of the kidney to concentrate urea, it was felt that the determination of the specific gravity of the

24. Wakefield, E. G.; Power, M. H., and Keith, N. M.: Inorganic Sulphates in the Serum in Early Renal Insufficiency, J. A. M. A. **97**:913 (Sept. 26) 1931.

urine, as outlined in the two hour test, would parallel rather closely the blood urea clearance. This expectation has not been fulfilled in every detail. In some cases, the urea clearance test shows a greater degree of renal impairment than could be surmised from the results of the two hour test. In other instances, determination of the specific gravity of the urine and the measurement of the night volume of urine suggested impairment of renal function while the urea clearance test gave normal values. In marked or maximal renal insufficiency, the two hour and urea clearance tests indicate the same degree of impairment.

The two forms of tests do not measure the efficiency of the same functions of the kidney. The urea clearance test gages the ability of the kidneys to eliminate urea. This may be successfully accomplished although only a small amount of urine is secreted within a given unit of time; that is, there is an impairment of the renal parenchyma as far as water excretion is concerned, as in the oliguric stage of acute diffuse glomerular nephritis. On the other hand, the elimination of urea may be normal although the power of concentration has been distinctly diminished, as is seen in the compensatory polyuria of certain stages of chronic nephritis. The tests of specific gravity furnish information as to the ability of the kidney to concentrate and to dilute; they indicate by what emergency and compensatory means the elimination of urea and of other solids is maintained. In the earlier stages of impairment of renal function occurring in chronic Bright's disease, each of these tests, the specific gravity and the urea clearance test, tells a story of its own. In the later stages, when all the functions of the kidney are more or less equally involved, both procedures will indicate a similar degree of diminution of renal function.

There are certain conditions that affect the specific gravity of the urine, and these must always be kept in mind when one is interpreting the results of the two hour test, which apparently do not influence the blood urea clearance. In Bright's disease with edema, the specific gravity of the urine will be increased or decreased by the deposition or the elimination of edema, respectively. In acute diffuse glomerular nephritis, the specific gravity of the urine often reaches surprisingly high and fixed figures. Irritation of the urinary tract, as caused by cystitis and pyelitis, tends to decrease the specific gravity of the urine. The urea clearance test is not markedly influenced by such factors.

The clearance test requires exactitude in collecting all the urine voided in a specified time, and this is often impossible in certain conditions of the bladder, notably retention of urine due to prostatic hypertrophy or incontinence from any cause. In such cases, the two hour test is of distinct advantage, since the urine need not be timed accurately. Again, the simplicity of the latter test is especially suited for the active hospital service or the physician's office.

In retrospect, it seems only fair at this time to emphasize again the necessity of investigating renal function from all possible angles, especially in early renal insufficiency. One or more of the tests or measures at the disposal of physicians may indicate slight impairment of renal function while others reveal normal renal function. It is obvious that the complexity of early functional pathologic conditions of the kidney is not determined by any single test. The urea clearance test is of considerable clinical value and should add materially to a better understanding of the function of the kidney as more work along such lines is accomplished.

SUMMARY

The urea clearance test usually demonstrates impairment of renal function before the urea, uric acid or creatinine is increased in the blood. Occasionally, cases of early renal insufficiency may show an elevation of the blood uric acid before the urea clearance test implies any impairment. In the chronic nephritides with definitely impaired renal function of some weeks' or months' duration, the degree of anemia parallels the amount of insufficiency as measured by the urea clearance test. The U/B ratio approximates the urea clearance values with urine rates less than 2 cc. per minute; it deviates appreciably from the standard clearance in oliguric patients; it bears no relation to the standard clearance with urinary rates of more than 2 cc. per minute. In cases of Bright's disease with less than 50 per cent of renal function as measured by the urea clearance test, the two hour test usually demonstrates impairment of renal function; occasionally, the two hour test may suggest slight renal insufficiency while the urea clearance test gives normal values; in other instances, the urea clearance test suggests graver degrees of impairment of renal function than can be surmised from determinations of the specific gravity and volume of the urine.

SEDIMENTATION TEST AS A ROUTINE LABORATORY PROCEDURE

OBSERVATIONS ON ELEVEN HUNDRED PERSONS

HERBERT J. SCHATTENBERG, M.D.
NEW ORLEANS

In 1918 Fahraeus,[1] in Germany, demonstrated the clinical importance of variable accelerated sedimentation of the erythrocytes in different conditions of disease. Westergreen,[2] also in Germany, carried out excellent work in this line; however, his technic is more elaborate and difficult, and his observations were chiefly on the sedimentation of red blood cells in tuberculosis. In America, Polak [3] is the most outspoken advocate of the sedimentation test. From its application in gynecologic conditions, he believes that this test offers another aid in the diagnosis of an infection which when frequently repeated and correlated with the clinical history, temperature curve and white blood cell changes forms a valuable index as to the time at which to operate and is also of prognostic value.

To explain the differences in speed of erythrocytic sedimentation, Fischl [4] and Schmitz [5] held that it is due to an alteration of the albumin-globulin ratio with an increase of the globulin. Kurten [6] expressed the belief that the increased sedimentation is due to an increased blood cholesterol. Fahraeus [1] stated that increased sedimentation is produced by an alteration in the electrical charge of the blood corpuscles, causing a loss of their repelling force and thereby bringing about their agglutination and more rapid sedimentation. Reyner [7] held that a difference in surface tension of the blood plasma is responsible for the phenomenon. He attempted to prove this by adding formaldehyde to the blood speci-

From the Department of Pathology, Tulane University.

These observations were made possible through the courtesy of the Dallas City Health Department and the Out-Patient Dispensary of Baylor Hospital.

1. Fahraeus, J.: Biochem. Ztschr. **89**:335, 1918.
2. Westergreen, A.: Brit. J. Tuberc. **15**:72, 1921.
3. Polak, J. O.: Clinical Significance of the Sedimentation Test, J. A. M. A. **90**:72 (Jan. 14) 1928.
4. Fischl, K.: Am. Rev. Tuberc. **10**:606, 1924.
5. Schmitz, H.: Am. J. Obst. & Gynec. **11**:353, 1926.
6. Kurten, H.: Arch. f. d. ges. Physiol. **185**:248, 1920.
7. Reyner, C. E.: The Sedimentation of Red Blood Cells, J. Lab. & Clin. Med. **14**:630 (April) 1929.

men to increase the surface tension and claimed that a decrease in red cell sedimentation happened in direct proportion to the amount of formaldehyde added. Hunt [8] felt that whatever is responsible for increased sedimentation is contained in the blood plasma, and that it is most likely an increase in the fibrinogen. Gradwohl [9] claimed that the hydrogen ion concentration influences sedimentation, since acidosis reduces and alkalosis accelerates the rate. This is due to the effect of these factors on flocculation and agglutination of the albuminous substances in the plasma.

TECHNIC

The value of any test depends on its simplicity of technic, its applicability and the additional information that it affords as compared with other diagnostic methods, or when correlated with them. A great number of different methods are used in making this determination. The two best known German methods are those of Westergreen [2] and Linzenmeier. The standard American method is that of Cutler,[10] which is a modified and simplified Westergreen technic. It calls for a small tube 5 mm. in diameter and marked in millimeters, beginning with 0 at the 1 cc. level and ending with 50 mm. at the bottom. One-tenth cubic centimeter of 3 per cent sodium citrate is first drawn into a hypodermic syringe to act as an anticoagulant; then 1 cc. of the patient's blood is drawn into the syringe. In order to facilitate the mixing of citrate and blood, a small bubble of air is drawn into the syringe. The syringe content is then emptied into a Cutler tube. A reading of the number of millimeters' drop in the red blood cell column is taken every ten minutes and recorded as shown in the chart.

It has been shown by the Cutler technic that in acute infectious processes the greatest fall in the red cells will take place in the first hour and that readings may be discontinued following the six readings at ten minute intervals. In this respect, the Cutler procedure has a great advantage over that of Linzenmeier, in which a mark is made on the tube at the 18 mm. level and a record made of the time when the blood cell column falls to this level, which may be in from thirty minutes to twenty-four hours. Naturally, the observer's time and attention are therefore focused on this procedure for a period varying from thirty minutes to twenty-four hours.

---

8. Hunt, H. F.: J. Lab. & Clin. Med. **14**:1061, 1929.

9. Schilling, V.: The Blood Picture and Its Clinical Use, translated by R. B. H. Gradwohl from 7th and 8th German editions, St. Louis, C. V. Mosby Company, 1929.

10. Cutler, in Nicholson, Daniel: Laboratory Medicine, Philadelphia, Lea & Febiger, 1930, p. 77.

THE VALUE OF THE SEDIMENTATION TEST IN VARIOUS CONDITIONS

It is my opinion that the sedimentation test will prove of great value if those physicians interested in medical diagnosis, preventive medicine, public health or life extension departments of insurance companies make the sedimentation test as a routine for all persons presenting themselves for physical examination. It is true with only a few exceptions, such as those of pregnancy and menstruation, that there can be an accelerated sedimentation rate only when there is a disease process or a destruction of tissue going on in the body. The test is not indicative of any one particular disease, but when the rate is markedly accelerated in a person who applies for a physical examination, it assures the examiner that there is trouble in this patient's body, and that he must reexamine him more carefully and more thoroughly to ascertain its exact nature.

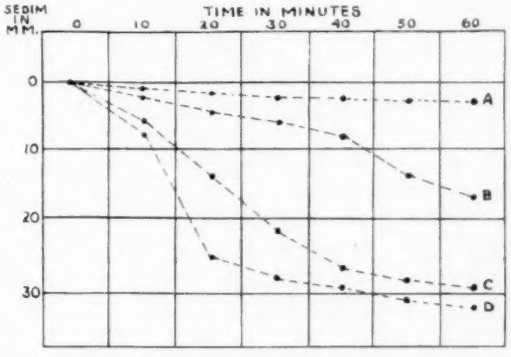


Chart after Nicholson (Laboratory Medicine, Philadelphia, Lea & Febiger, 1930, p. 80). *A* represents a normal curve; *B*, a clinically quiescent process; *C*, a clinically slightly active process, and *D*, a clinically markedly active process.

With the foregoing considerations in mind, and knowing how much this test would help the examiner in obscure and latent stages of various diseases, my associates and I decided to make the test as a routine for applicants presenting themselves for examination for the purpose of obtaining health cards in the Dallas City Health Department. Tests were made also on patients in the outpatient dispensary of Baylor Hospital. The results obtained have been interesting. In every person presenting an accelerated sedimentation we have been able to find the cause in the form of some disease process. In all, eleven hundred persons were tested for their sedimentation rates. Since all applicants received also a routine Wassermann blood test, there was an excellent opportunity to see what the sedimentation test showed in the persons whose blood gave a positive Wassermann reaction. Approximately 40 per cent of these showed accelerated red cell sedimentation, and in all

of the latter there was shown some form of open lesion or a very active syphilitic process. Those whose blood gave a positive Wassermann reaction without an accelerated red cell sedimentation appeared to have latent syphilis, or showed no active clinical or outward appearance of syphilis.

Besides the persons described with positive Wassermann reactions and markedly accelerated sedimentation rates in whom open or active syphilitic lesions were found, six additional ones were found with rapid sedimentation rates. On further and more thorough clinical examination of these six, one was found to have myeloid leukemia, one pellagra, one incipient pulmonary tuberculosis, two subacute salpingitis and one pityriasis rosea. The conditions named were discovered only after accelerated sedimentation rates had focused further attention on these patients. From this it may be seen what an aid one has in this test when it is made as a routine for all persons presenting themselves for physical examination.

In gynecology, the sedimentation test may indicate the best time at which to perform a nonurgent operation. A patient showing a sedimentation rate of over 18 mm. in an hour, if operated on for pelvic disease, is apt to have a stormy convalescence. A reading of 18 mm. in thirty minutes is easily found in cases of acute pelvic abscess, and if the patient is operated on, conservative evacuation should be employed. In the differentiation between ectopic pregnancy and acute exacerbation of a chronic inflammatory process, a rapid sedimentation points toward infection, while a slow sedimentation indicates ectopic pregnancy. With cysts or uterine fibroids, the sedimentation is normal, provided there is no complicating infection or degenerative change in the cyst or fibroid. The sedimentation test is valueless after the third month of pregnancy and for the first two weeks of the puerperium, as then readings cannot be attributed to infection. Furthermore, after the fourth month of pregnancy there are so many clinical and physical signs to assist in the diagnosis of pregnancy that there is no need to use sedimentation. In pelvic infection, the sedimentation test is far more reliable than leukocytic counts. It is well known that leukocytic counts may fluctuate from high to low levels during a twenty-four hour period; consequently, there is danger of the surgeon's taking a blood specimen when the fluctuation is at a low level and considering that the time for operation is at hand, whereas, if the count is repeated in several hours, it will be found again dangerously high. This error never happens with the sedimentation test. When the sedimentation rate indicates an active process, the rate can be shown to return to normal slowly and gradually as the patient's condition improves. Irregular fluctuations do not occur with this procedure as with leukocytic counts. In fact, Polak believes that the sedimentation test never yields erratic results. Furthermore, he is of the opinion that this test is the best indicator as to the time most suitable for

the patient's discharge from the hospital. Polak stated, "Previously, we have felt safe in discharging a patient when she had a normal temperature for one week and a normal leukocyte count, even in the presence of pelvic exudate. Many of these types of patients on returning home and assuming their usual household and social duties have been seized with severe pelvic pain and rise in temperature. These women have returned with increased exudate and tenderness and in need of immediate hospitalization. All this could have been avoided had the patient been kept in the hospital until the sedimentation time had returned to normal."

In tuberculosis, Cutler [10] considers the sedimentation rate of more value in estimating the activity of a tuberculous process than the pulse rate, temperature or weight. The pulse rate and the temperature may return to normal while the process is still more or less active. The sedimentation rate does not return to normal until the tuberculous lesion is well encapsulated; therefore, this test should be run as a routine in hospitals for tuberculosis on all patients at intervals of a month or oftener to keep a check on their progress.

SUMMARY

1. A standard technic for the performance of the sedimentation test should be accepted. There are too many modifications of the sedimentation test extant. That of Cutler is the most applicable, since it gives one the benefit of the first hour's readings, which are the most important, at ten minute intervals. Furthermore, the observer's time is taken up for a period of one hour only.

2. A normal sedimentation rate with but few exceptions rules out the presence of disease. Since there is a physiologic increase in fibrinogen during menstruation and pregnancy, one naturally expects a rapid sedimentation of red blood cells to occur.

3. The sedimentation test is useful in the differential diagnosis of gynecologic conditions.

4. It is useful in determining the proper time for nonurgent elective operations, in prognosticating postoperative complications after the first week and as a criterion for discharging patients.

5. In active tuberculosis, the sedimentation rate is always rapid, regardless of physical findings; it is also of more value than are the pulse rate, temperature and weight in checking treatment in a tuberculous patient.

6. In health departments, it assists in the diagnosis of obscure diseases that would otherwise escape detection. Accelerated sedimentation means destruction of tissue, and the examiner therefore reexamines the patient to ascertain the nature of the pathologic process that was overlooked the first time.

METHEMOGLOBINEMIA

WILLIAM J. DIECKMANN, M.D.
CHICAGO

Methemoglobinemia occurring spontaneously is rare, and a case of twenty-seven years' duration is, so far as I know, unheard of. The first cases were described in 1902, by Stokvis [1] and Talma,[2] who considered the cyanosis to be due to methemoglobin, but van den Bergh,[3] in 1905, in a study of two cases, was able to distinguish two types of this condition, which had been known and is still known as "enterogenous cyanosis." He showed that the cyanosis was due in one of his cases to sulphemoglobin and in the other to methemoglobin circulating in the blood. Up to that time there had been no differentiation between the two types, and some doubt, therefore, arises as to the true nature of the cases described by Stokvis and Talma. Excluding their four cases because of this doubt, I have been able to collect the following reports of six cases of methemoglobinemia of unknown origin.

REPORT OF CASES

Van den Bergh [3] (1905).—A man, aged 25, complained for seven years of weakness, cyanosis, edema and intermittent diarrhea and constipation. A milk diet gave only temporary improvement.

Van den Bergh and Grutterink [4] (1906).—Case 1: A man, aged 34, stated that for four years he had had dysentery, headache, a feeling of paralysis of the legs and arms and cyanosis.

Case 2: A man, aged 51, gave a similar history.

Gibson and Douglas [5] (1906).—A woman, aged 36, complained of weakness, headache, cyanosis and diarrhea of three years' duration, together with a change in complexion and color of the hair. There had been free use of aniline derivatives, but the cyanosis persisted when the drugs were stopped. Nitrites were present in both the saliva and the blood. Bacillus coli was recovered in pure culture from the blood, and presumably was a factor in the production of the "microbic cyanosis," as it was called by the authors.

From the Department of Obstetrics and Gynecology, Washington University, School of Medicine, the St. Louis Maternity Hospital, and the Department of Obstetrics and Gynecology, University of Chicago.

1. Stokvis, B. J.: von Leyden's Festschrift, Internat. Beitr. z. inn. Med. **1**:597, 1902.
2. Talma, S.: Berl. klin. Wchnschr. **39**:865, 1902.
3. van den Bergh, A. A. H.: Deutsches Arch. f. klin. Med. **83**:86, 1905.
4. van den Bergh, A. A. H., and Grutterink, A.: Berl. klin. Wchnschr. **43**:7, 1906.
5. Gibson, T. A., and Douglas, C. C.: Lancet **2**:72, 1906.

LLOYD[6] (1924).—A nurse, aged 26, was admitted to the hospital because of attacks of abdominal pain, associated with cyanosis. She dated the onset of her condition to a strain caused by lifting a patient. She was seen at the hospital at irregular intervals for four years, and although several laparotomies were performed, including resection of the colon, there was no permanent relief. The blood, which at first contained methemoglobin, soon contained sulphemoglobin instead. A report of the same case in 1928 by Waterfield[7] showed that at this time the blood contained both methemoglobin and sulphemoglobin. The patient eventually died. The total duration of the cyanosis was seven years, of which time more than two years were spent in the hospital.

MILLER[8] (1930).—Enterogenous methemoglobinemia occurred in a 9 year old girl. The foster mother, who had obtained the child at 9 months of age, stated that she was blue at that time and had been blue from birth. The color of the hair varied with the degree of cyanosis. Methemoglobin was demonstrated by spectroscopic examination, and although the child was followed for two years there was no improvement. Some relief resulted from colonic irrigations with a 1:4,000 solution of potassium permanganate. It was also noted that an increase of indican in the urine was always followed within from twenty-four to forty-eight hours by cyanosis.

AUTHOR'S CASE.—N. B., a white woman, aged 27, was admitted to the hospital on Feb. 16, 1931, because of cyanosis. She stated that her skin had been blue from birth, with an increase in intensity during pregnancy. Her husband stated that the color had been the same since he first met her. Several years before admission, she took "bromoseltzer," and for the past three months, she had taken 10 grains (0.65 Gm.) of acetylsalicylic acid daily because of "neuralgia in her teeth." At the time of admission she was pregnant for the third time, and she was at term on March 16, 1931. Both previous pregnancies had been terminated by delivery with forceps because of "weak pains." The first baby was stillborn, and the second died at the age of 3 months.

The note of the cardiologist was as follows: "The patient has been blue all her life, but has never had any symptoms of cardiac impairment. There is cyanosis of the fingers, nose and face, especially around the nose. There is no clubbing of the fingers. The heart shows no enlargement on percussion. There is a systolic murmur in the third left interspace. The pulse rate is 120. There is no evidence of organic or acquired heart disease, but with the tachycardia and cyanosis of long standing, congenital heart disease is quite likely." The electrocardiogram showed tachycardia.

Blood was obtained for a determination of oxygen saturation, and was also used for a routine chemical analysis. As a routine hemoglobin was determined as carboxyhemoglobin (colorimetrically), and in this case the technician noted that the unknown carboxyhemoglobin solution could not be made to match the standard satisfactorily. When the hemoglobin was determined by the oxygen capacity method, only 8.8 Gm. was found, in contrast with 11 Gm. when it was determined as carboxyhemoglobin. The discrepancy was even greater when it was determined as acid hematin. The marked variations in hemoglobin, as determined by different methods, indicated an abnormal form of hemoglobin, and spectroscopic examination showed a large amount of methemoglobin. This compound does not combine with oxygen or carbon monoxide, and the higher reading with the colorimetric determination as carboxyhemoglobin was due to the presence of

6. Lloyd, N. L.: Guy's Hosp. Rep. **74**:376, 1924.
7. Waterfield, R. L.: Guy's Hosp. Rep. **78**:265, 1928.
8. Miller, R.: Arch. Dis. Childhood **5**:73, 1930.

methemoglobin. The determination of acid hematin gave both hemoglobin and methemoglobin, but again the results would not have been questioned had it not been for the determination of oxygen capacity.

On February 27, the cardiologist stated that "because of the practically negative heart findings and the presence of methemoglobinemia, a diagnosis of the latter is more likely than one of congenital heart disease."

On March 3, the patient went into labor, and because there was only 7 Gm. of functioning hemoglobin per hundred cubic centimeters of blood, she was given 600 cc. of citrated blood. The blue color almost disappeared following the transfusion and was much less noticeable up to her discharge on March 17, 1931. She was delivered of a 3,700 Gm. normal baby after an easy seven hour labor. The erythrocyte count varied from 4,330,000 to 5,110,000. The pulse rate ranged from 80 to 100 ante partum and from 70 to 90 post partum.

During the period in the hospital everything was done to prevent the ingestion of drugs. Cultures made from the mouth and stools were negative for the nitrosobacillus. Purgation had no effect on the cyanosis.

The patient was seen on April 14; she was again cyanotic. Examination of the blood showed that methemoglobin was present in large amounts. The essential data have been tabulated (table).

Results of Determination of Hemoglobin

	Hemoglobin (Gm.) Determined as					
Date	Oxygen Content	Oxygen Capacity	Carboxy-hemoglobin	Acid Hematin	Hematocrit, per Cent	Methemoglobin, Spectroscopic
2/19/31	7.2	8.8	11.0	12.7	34	Large amount
2/24/31	7.7	8.6	10.4	11.9	34	Large amount
2/25/31	....	....	V-10.0	....	36	Large amount
3/ 3/31	Delivered—blood transfusion 600 cc.					
3/12/31	....	V*-11.9	12.9	....	42	Faint trace
4/14/31	8.9	10.6	11.3	14.2	38	Large amount

* V = Venous.

Clinically, methemoglobinemia is reported most frequently in factory workers, whose skins or lungs come into contact with aromatic coal tar derivatives, such as aniline, nitrobenzene and the nitrophenols. Reports of cases of methemoglobinemia due to the excessive use of acetanilid are also rather common, but the occurrence of the condition spontaneously is rare, and the etiology is unknown. Van den Bergh and Grutterink stated that they had found nitrites in the blood and assumed that they were the cause of the condition. However, the origin of the nitrites has not been determined, although in 1913 Mackenzie-Wallis [9] found in the saliva of four patients suffering from sulphemoglobinemia a nitrite-producing bacillus. He also found that the serum of each patient contained a substance capable of reducing oxyhemoglobin to hemoglobin, and he therefore suggested that the sulphemoglobinemia depended on the absorption of nitrites from the saliva and of small quantities of hydrogen sulphide from the colon. His results have not been confirmed.

9. Mackenzie-Wallis, R. L.: Quart. J. Med. **7**:73, 1913.

The outstanding feature of methemoglobinemia is cyanosis and, if the condition is severe, dyspnea. The cyanosis is due to the dark brown color of methemoglobin and appears to be more intense than cyanosis produced by a similar concentration of reduced hemoglobin in the blood. The dyspnea is referable to the anoxemia caused by diminution in the oxygen-carrying capacity of the blood.

The discovery of methemoglobin in the case reported was accidental, but the possibility of its occurrence in all cases of cyanosis should be kept in mind. The diagnosis of methemoglobinemia was based on the following spectroscopic examination: The citrated blood was diluted with nine volumes of water and examined spectroscopically. A band centering at 638 Mu Mu was found. Solutions of methemoglobin gave a similar absorption band. The band disappeared on the addition of sodium hydrosulphite. This excludes the possibility that the pigment was sulphemoglobin.

The usual purpose of a determination of hemoglobin is to ascertain the concentration of a functional blood pigment, that is, pigment which carries oxygen. Since no known hemoglobin derivative exhibits this property, the presence of such substances as methemoglobin, carbon monoxide hemoglobin and sulphemoglobin in blood, when knowledge of the concentration of hemoglobin alone is desired, leads to erroneously high results with all colorimetric methods.

In the Newcomer method, methemoglobin, like hemoglobin, is converted into acid hematin by hydrochloric acid. The same interference occurs in other methods involving the formation of acid hematin (e.g., the Sahli method).

In the Palmer method, methemoglobin interferes with the determination merely because of its tinctorial powers; it does not react with carbon monoxide.

The presence of methemoglobin in blood may vitiate determinations of oxygen content and capacity (with the Van Slyke procedure) unless certain precautions are observed (the "inactive" pigment does not interfere with the determination per se). Warburg [10] and his co-workers recently reported that mammalian red blood cells treated with amyl nitrite (to convert part of the hemoglobin to methemoglobin) when subsequently incubated with dextrose or lactic acid undergo the following changes: Methemoglobin oxidizes the sugar or lactate and is thereby converted back to hemoglobin. This then picks up oxygen to form oxyhemoglobin. In rabbit's blood regeneration of hemoglobin is rapid; in dogs and in human blood it is less so. Wendel [11] has confirmed and extended these findings.

10. Warburg, O.; Kubowitz, F., and Christian, W.: Biochem. Ztschr. **227**:245, 1930.

11. Wendel, W. B.: Proc. Soc. Exper. Biol. & Med. **28**:401, 1931

The bearing of these phenomena on determinations of oxygen capacity and content is obvious. If blood containing methemoglobin is allowed to stand before the oxygen capacity is determined, the value of this may be significantly increased over that which would have been obtained had the determination been carried out immediately after the sample was drawn. Chilling slows the reaction, but does not inhibit it completely.

On March 12, the glycolytic activity of the blood cells was found to be normal. From this it would seem probable that the patient's blood would rapidly reduce methemoglobin to hemoglobin. The constancy of the methemoglobinemia, then, is probably due to the continued presence or production of a substance capable of oxidizing hemoglobin.

Further experiments were contemplated in the hope that a cause for the formation of the methemoglobin could be found, but the patient could not be persuaded to return to the clinic.

SUMMARY

A case of cyanosis due to methemoglobin of unknown origin, which had been present for twenty-seven years, is reported. The accidental discovery in a routine examination warrants the statement that the condition would be detected more frequently if the blood of cyanotic patients were examined spectroscopically. More attention should be given to determination of the oxygen capacity of the blood than to the colorimetric determination of hemoglobin. In the normal person, the results are identical, but in certain pathologic conditions the apparent hemoglobin may be within normal limits and yet the patient be suffering from anemia because a portion of the hemoglobin cannot carry oxygen.

The spectroscopic examinations and the determination of the glycolytic activity were performed by Mr. William B. Wendel in the Laboratory of Biological Chemistry, Washington University.

A METHOD FOR THE SIMULTANEOUS ENUMERATION OF BLOOD PLATELETS AND RETICULOCYTES

WITH CONSIDERATION OF THE NORMAL BLOOD PLATELET COUNT IN MEN AND IN WOMEN

WILLIAM DAMESHEK, M.D.
BOSTON

The methods so far introduced for the enumeration of the blood platelets are well-nigh legion. Their very number speaks for a lack of standardization in technic. The method presented here has the following advantages: 1. The individual blood platelets, which are kept from clumping, can be seen, studied and counted without difficulty. 2. The reticulocytes can be counted in the same preparation.

The enumeration of the blood platelets becomes important in the study of the hemorrhagic diseases, particularly in purpura hemorrhagica; it is also of great interest and probably of more value than is realized, in the study of the anemias, leukemias and associated disorders. Since the blood platelets are derived from the megakaryocytes of the bone marrow, they should give, in association with a study of the number of reticulocytes and neutrophils, a complete index as to the activity of the marrow.

The chief difficulty encountered in the enumeration of the blood platelets depends on the marked tendency of these bodies to clump together. This difficulty has often led to neglect of this valuable laboratory procedure. Despite this, counting methods have been numerous, although as yet no completely satisfactory procedure has been devised.

There are in general two main methods for the estimation of the blood platelets. The first is the direct or counting-chamber method, in which the blood platelets are enumerated in much the same way as are the red blood cells. The methods of Wright and Kinnicutt [1] and of Buckman and Hallisey [2] are examples of this technic. The second general method is the indirect one, in which the proportion of platelets to red blood cells is determined in a blood smear preparation. The

From the Medical Clinic and the Department of Pathology, Beth Israel Hospital.

1. Wright, J. H., and Kinnicutt, R.: A New Method for Counting Blood Platelets for Clinical Purposes, J. A. M. A. **56**:1457 (May 20) 1911.

2. Buckman, T., and Hallisey, J. E.: Studies in Properties of Blood Platelets, J. A. M. A. **76**:427 (Feb. 12) 1921.

absolute number of platelets per cubic millimeter is then calculated after the determination of the red blood cell count. The methods of Fonio [3] and Hittmair [4] are examples of this technic. The estimation of the number of blood platelets, (whether normal, increased or diminished) by inspection of fixed blood smear preparations may be included among the indirect methods, although obviously it is not to be relied on for more than a rough approximation.

The various disadvantages of the direct or hemacytometer method have been cited by Olef [5] in a recent article. Among them may be mentioned the following: clumping of the platelets during the drawing of blood into the pipet, the impossibility of visualizing the smallest platelets with the high dry objective, the "loss" of some of the platelets in the 100 microns (0.1 mm.) space that exists between the level of the counting chamber and the cover glass above it, the sticking of platelets to the sides of the pipet or to the parts of the hemacytometer, and, finally, (when a stain is used) the precipitation of the stain and the resultant confusion of granules of the stain with blood platelets. Attempts have been made to surmount some of these difficulties by various means, without much success. The direct method, although apparently simple in its application, has many inherent sources of error.

The indirect method has been practiced in several different ways. The simplest and least accurate is the estimation of the blood platelets from the ordinary stained blood smear. This suffices for the routine study of blood smears, but it is worthless when an exact figure for the number of the blood platelets is of importance. Numerous methods are used in the counting of the blood platelets indirectly. Those of Fonio,[3] Hittmair [4] and Olef [5] may be cited as examples. Each of them appears, however, to have certain shortcomings, which we have attempted to overcome.

In the method of Fonio,[3] a drop of 14 per cent magnesium sulphate is placed on the finger, which is then punctured through the drop with a lancet. Cover slip preparations are made in the ordinary way by drawing two cover slips apart, and they are stained with Wright's stain. The blood platelets, which swell when they come in contact with the magnesium sulphate solution, are easy to enumerate. However, the act of drawing apart the cover slips in making the blood smear often causes a marked unevenness in the distribution of the platelets.

3. Fonio, A.: Ueber ein neues Verfahren der Blutplättchenzählung, Deutsche Ztschr. f. Chir. **117**:176 (June) 1912.

4. Hittmair, A.: Die Blutplättchen, Folia haemat. **35**:156, 1928.

5. Olef, I.: Blood Platelets: An Improved Indirect Method for Their Enumeration, Arch. Int. Med. **46**:585 (Oct.) 1930.

thereby making the method almost as inaccurate as an estimation from fixed smears stained with Wright's stain.

In the method of Hittmair,[4] a drop of blood is placed on a cover slip that has already been prepared with a dried film of brilliant cresyl blue. The cover slip is then dropped on a slide and examined under oil immersion lens. There is no provision, however, for preventing the platelets from clumping. In addition, their distribution is usually very uneven.

In the method of Olef,[5] extravagant precautions are taken to prevent the platelets from clumping. The drop of blood is allowed to fall into a small paraffin cup which contains a solution of sodium metaphosphate. A paraffin-coated wooden applicator is used to stir the resultant mixture, and a drop of this diluted blood is transferred to a slide over which is placed a cover slip. The blood platelets are then counted, the oil immersion lens being used. No stain is used. This method is accurate, but it has two distinct disadvantages: 1. It involves the use of a special apparatus, however simple. 2. There is no provision for staining the blood platelets, some of which may be missed.

METHOD

This is an indirect method in which the diluting fluid is not only isotonic but contains brilliant cresyl blue for staining purposes and sodium citrate for use as an anticoagulant. This solution, which is a modification of the one devised by Buckman and Hallisey[2] for counting the platelets directly, contains the following ingredients:

	Gm. or Cc.
Brilliant cresyl blue	0.15
Sodium citrate	0.40
Sucrose	8.00
Water	100.00

The cane sugar and the sodium citrate are dissolved in distilled water to which is then added the brilliant cresyl blue. The resulting solution is mixed well and filtered. Three drops of a solution of formaldehyde (1:10) U. S. P., are added as a preservative.

This solution keeps well in a cold place, but it should be filtered every three or four weeks. It has the following advantages: 1. The stained platelets are easily seen. 2. The stained network and granules of the reticulocytes are well brought out. 3. The anticoagulant prevents clumping of the platelets. 4. The isotonicity of the solution aids in the proper separation and distribution of the blood cells.

One of the fingers is well cleaned with alcohol or acetone and then dried. A puncture wound is made. The first drop of blood is discarded. A fairly large drop (about 3 mm. in diameter) of the staining solution is placed over the puncture wound, and the finger is gently squeezed so that a small amount of blood wells up into the drop of staining solution. The correct amount of solution to be placed on the finger and the proportion of blood to be squeezed into it can be learned only with experience. The proportion of blood to stain should be small (about 1:5) so that a well-spread preparation, not overcrowded with red blood cells, is obtained. The

mixture of blood and stain is immediately transferred to a cover slip, which is then dropped on a slide. Cleanliness of glassware is essential.[6]

The preparation is examined under oil immersion lens, preferably after from fifteen to forty-five minutes to permit complete staining of the platelets and reticulocytes. Counts may be made at any time within two hours. Counts made up to four hours, if the preparations are ringed with petrolatum, are also accurate. Permanent preparations cannot be made with this method. Blood platelets, even those measuring only 1 micron or less in diameter, are easily seen as highly refractile opalescent bodies taking a pale bluish stain. Reticulocytes are well stained, even the slightest degree of granulation and reticulation being easily seen. The rapid motion of some of the reticulocyte granules can easily be followed. White blood cells, which also take the stain, may easily be recognized and counted if desired.

One thousand red blood cells are counted (the microscopic field being cut down if desired by the insertion in the eyepiece of an appropriately perforated paper disk), and the number of platelets and reticulocytes seen during this enumeration is recorded. The *number of reticulocytes* is expressed as a percentage of the total number of red blood cells. The *absolute number of platelets* is obtained by: (1) performing a count of the red blood cells, and (2) solving the following equation: red blood count : 1,000 :: platelets per cubic millimeter : platelets counted. This is most conveniently done by multiplying the first four figures of the red blood cell count by the number of platelets seen in counting 1,000 red blood cells. Thus, if in counting 1,000 red blood cells, 20 reticulocytes and 200 platelets are seen, and the red blood cell count is 3,000,000 per cubic millimeter, the percentage of reticulocytes is 2; and platelet count is 3,000 times 200, or 600,000 per cubic millimeter.

RESULTS

The method has been used as a routine in the blood laboratory during the past three years. It has proved simple in its application and easy to teach to technicians and medical students. Particularly satisfactory have been the ease with which the platelets are seen and studied and the obtaining of reticulocyte counts in the same preparation.

RETICULOCYTES

The reticulocytes as seen with this method are unusually well stained, in fact, far superior to those seen in the "wet" method which was described by me in 1926.[7] Numerous comparison counts of reticulocytes were made by the present method and other methods commonly in use. In each instance, the reticulocyte count by this method was at least as high as with other methods and frequently higher. It was felt that the higher counts obtained were due to the

6. The use of new slides and cover slips is essential. It is advisable to keep them from one to three days in a cleaning solution of sulphuric acid, U. S. P., and potassium bichromate, after which they are thoroughly rinsed in water and kept in an 80 per cent solution of alcohol ready for drying.

7. Dameshek, W.: The Reticulated Red Cells—Their Clinical Significance, Boston M. & S. J. **194**:659 (April 29) 1926.

ease with which even the slightest degree of reticulation could be studied. The last stage in reticulation, the highly refractile granule described by Isaacs[8] could readily be determined. Although Heath and Daland[9] recently reported that a large number of substances, among them sucrose (a constituent of our diluting solution), inhibited the staining of reticulocytes, it was impossible to demonstrate such inhibition in the present study.

BLOOD PLATELETS UNDER NORMAL CONDITIONS

Morphologic Aspects.—Normally, the platelets vary in size from 0.5 to 2 microns and are round, oval or crescentic. They are highly refractile, apparently "solid" and biconvex, in contrast to the biconcave shape of the red blood cell. The smallest of them appear to have a definite, though slight, motility which may be brownian in type. The edge of the platelet is usually "fuzzy," owing to numerous spinelike processes that protrude at all points about the periphery. These processes frequently become greatly lengthened, especially if the preparation is allowed to stand for an hour or more. When two platelets are seen to float near each other, these elongated processes become entangled, causing the platelets to clump together. This appears to be the mechanism by which agglutination of platelets takes place. At either end of some of the larger platelets a hyaline unstained "ectosarc" is frequently observed. The center of the platelets is usually occupied by blue-staining material arranged in granules and, at times, in larger masses. This is not a constant finding and is usually absent in the smallest platelets. At times, the central stained mass resembles a nucleus. Further observations on the morphology of the blood platelets are being made.

Quantitative Aspects.—One hundred platelet counts on supposedly normal persons with normal hemoglobin and red blood cell count were made. Fifty-two men and forty-eight women were first studied. When the results were plotted, the curve for men showed a normal frequency (chart 1), whereas that for women was irregular.

The mean blood platelet count found for men was 716,000 per cubic millimeter; the median count, 710,000 per cubic millimeter. Fifty-five per cent of the counts were between 600,000 and 800,000 per cubic millimeter, and 84 per cent between 500,000 and 900,000 per cubic millimeter. The normal range for men is probably represented by the latter figures.

8. Isaacs, R.: The Refractive Granule Red Blood Corpuscle: Its Behavior and Significance, Anat. Rec. **29**:299, 1925.

9. Heath, C. W., and Daland, G. A.: Staining of Reticulocytes by Brilliant Cresyl Blue: Influence of Solutions of Substances, Arch. Int. Med. **48**:133 (July) 1931.

Since the frequency curve for presumably normal women selected at random was irregular, it was felt that some variable factor previously ignored might be present. Because of the well known relationship between blood platelets and hemorrhage, the possibility that the

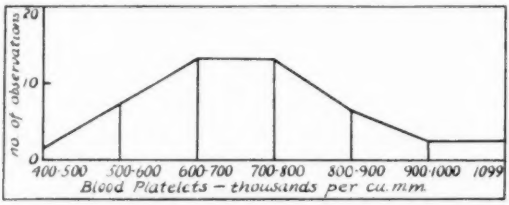


Chart 1.—The range of blood platelet counts in normal men.

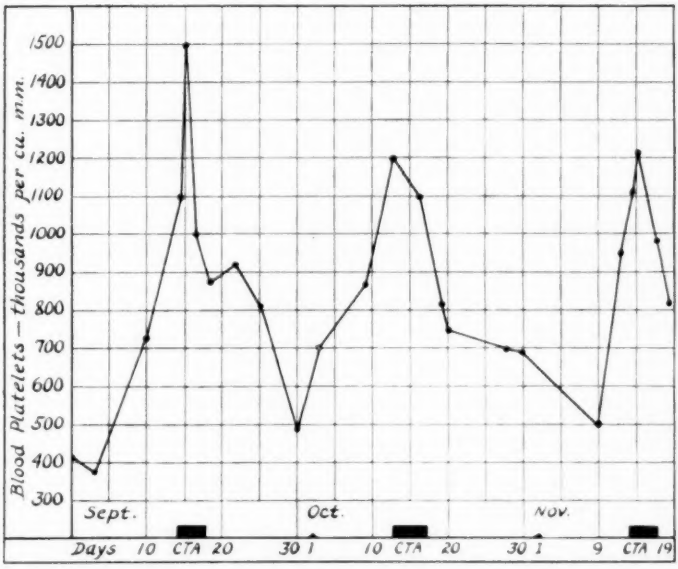


Chart 2.—*A.G.*, variations in blood platelet count with menstruation; *CTA*, menstrual flow.

menstrual cycle might have some influence on the blood platelet count immediately suggested itself. A series of observations was accordingly made on a small group of normal women at frequent intervals. It was soon found that the counts varied markedly with the menstrual cycle (charts 2, 3 and 4), becoming extremely high on the first day of the period, remaining elevated for from three to five days and then

gradually becoming depressed to a fairly constant intermenstrual figure, which varied with the individual subject. On the appearance of the menses, there was always a sudden and marked rise in the blood platelet count, figures of from 1,000,000 to 1,500,000 being usual. The peak of the count was usually reached on the second or third day, after which there was a gradual decline to the intermenstrual level. As comparable controls, frequent platelet counts made on a small group of normal men and on a few nonmenstruating women showed little or no variation from count to count.

Further study of the platelet counts in women is now in progress. At present, normal and mean figures for women cannot be given. However, if one takes the fairly constant intermenstrual figures as representing the normal blood platelet count, the range at this time is usually between 400,000 and 600,000 per cubic millimeter. These

TABLE 1.—*Average Number of Blood Platelets Per Cubic Millimeter in Normal Persons as Determined by Various Methods*

Direct Methods		Indirect Methods	
Wright and Kinnicutt[1]	297,000	Fonio[3]	234,000
Buckman and Hallisey[2]	300,000	Pratt (J. A. M. A. **45**: 1999 [Dec. 30] 1905)	469,000
Casey and Helmer (Proc. Soc. Exper. Biol. & Med. **27**: 965, 1930; **28**: 523, 1931)	536,000	Olef[5]	619,000

figures probably represent the normal range in women, the menstrual cycle being set aside.

It is probably better to cite normal ranges both in men and in women than to give average figures for a mixed group, as is usually done in most of the published papers. Average normal platelet counts as reported by other investigators are usually lower than with this method, although with his careful indirect technic, Olef has obtained counts in the same range. The normal platelet counts as determined by other methods are given in table 1.

The blood platelet count as determined by this method was compared in about two thousand instances with that determined by the direct method of Buckman and Hallisey. The normal range with the latter method is from 200,000 to 400,000 per cubic millimeter. Higher counts were always obtained with the present method, and usually the counts were twice as high. It was felt that these higher counts were probably more accurate, since they represented the finding of a larger number of platelets per unit of number of red blood cells than with the other method. The implication is present that when direct methods are used, not all of the platelets are counted.

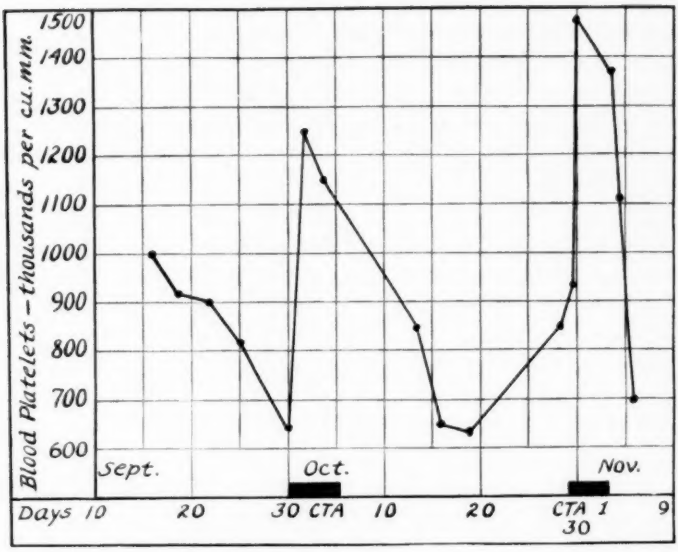


Chart 3.—*C.D.*, variations in blood platelet count with menstruation; *CTA*, menstrual flow.

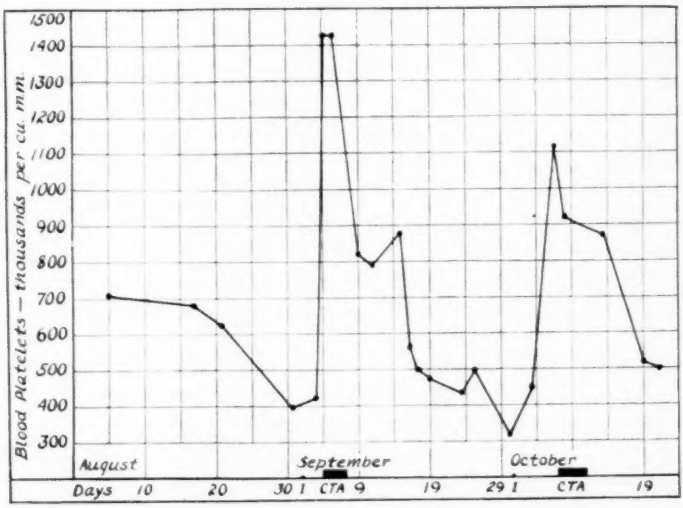


Chart 4.—*S.G.*, variations in blood platelet count with menstruation; *CTA*, menstrual flow.

Accuracy of the Method.—Various procedures were used to check the accuracy of the method previously outlined. These are as follows:

(*A*) Comparison of Blood Platelet Counts Obtained in Different Preparations of the Same Blood and in Different Fields of the Same Preparation: At times, as seen from table 2, there was rather marked variation in the count of the blood platelets in different parts of the same preparation, the difference being possibly as great as 70,000 per cubic millimeter. This was in all probability due to the unequal distribution of the blood platelets, which at times took place. Absolute cleanliness of glassware is essential in preventing this variation. In the presence of a normal erythrocyte count, a difference in 10 platelets per thousand red blood cells causes a difference in the platelet count of 50,000 per cubic millimeter. The distribution of the blood platelets should therefore be approximately equal throughout the preparation. It is my custom always to make at least two preparations and count different parts of

TABLE 2.—*Comparison of Platelet Counts in Different Preparations of Same Blood and Different Fields of Same Preparation*

	Red Blood Cells (Millions)	Upper Half "A" (Thousands)	Lower Half "A" (Thousands)	Upper Half "B" (Thousands)	Lower Half "B" (Thousands)
J. A.	5.40	818	838	868	812
S. G.	5.01	554	574	578	592
R. L.	4.33	468	406	438	428
B. S.	5.64	804	778	826	850
E. N.	1.75	198	174	190	186

the same preparation, obtaining thus an average count of the platelets per thousand red blood cells. If one preparation shows an uneven distribution of the blood platelets, it is discarded, only the satisfactory one being used.

The error in the technic is probably not more than 70,000 per cubic millimeter (about 7 per cent) when the erythrocyte count is high and not more than from 10,000 to 20,000 when the count of the red blood cells and blood platelets is much reduced. With perfection in technic, the percentage of error may further be reduced.

(*B*) Comparison of Leukocyte Counts by Direct and Indirect Methods: Before blood-counting pipet and hemacytometers were devised, the number of leukocytes was routinely compared with a large number of red blood cells in stained or unstained smears. Normal and abnormal proportions were thus determined. At present, however, the direct method of counting white blood cells in the hemacytometer is universally accepted as accurate. It was felt that the old indirect method could be applied to white blood cell counting and that both methods should give comparable results, especially since there is no error due

to clumping of leukocytes in the pipet (direct method). A comparison of these two methods might therefore be utilized to check the accuracy of the indirect platelet method, especially in patients with high leukocyte counts. The aforementioned technic for the enumeration of the blood platelets was accordingly used, the white blood cells being counted in the same preparation. A few of these results are given in table 3.

As was expected, only little variation was found between the leukocyte count as determined by the undoubtedly accurate pipet method and that obtained in the indirect fashion used to obtain counts of the platelets. On the other hand, there was much irregularity and wide differences between the methods used for the enumeration of the blood platelets, owing, in all probability, to the reasons already enumerated.

TABLE 3.—*Comparison of Leukocyte Counts by Direct and Indirect Methods*

		Red Blood Cells (Millions)	White Blood Cells (Thousands) Pipet Method	White Blood Cells (Thousands) Author's Method	Blood Platelets (Thousands) Author's Method	Blood Platelets (Thousands) Buckman and Hallisey Pipet Method
P. A.	Acute myelogenous leukemia	2.96	275	296	186	66
		2.88	236	252	178	66
		2.22	248	282	99	68
		2.23	324	312	145	70
		1.88	482	510	54	39
H. L.	Chronic lymphatic leukemia	2.40	180	196	132	110
		2.67	208	252	235	208
		2.94	318	312	258	102
		2.76	172	196	195	102
		2.78	146	152	222	120
		2.83	108	106	223	106
		2.65	52	68	216	114
		3.17	64	68	263	180
E. H.	Ulcerative colitis	4.16	25	24	807	404
		3.88	17	17	788	326

(*C*) Correlation with Clinical Data: This new method of platelet counting, by its accuracy, has permitted careful study of a large group of pathologic conditions, and these findings will be elaborated on in a later communication. It may be said that in certain diseases in which there was either progressive decrease or increase in the blood platelets, there was exact correlation with the progress of the disease.

SUMMARY AND CONCLUSIONS

1. An indirect method for the simultaneous enumeration of the blood platelets and reticulocytes is described. It depends on the use of an isotonic, anticoagulating solution containing the "vital" dye brilliant cresyl blue. The platelets are examined under oil immersion lens. Reticulocytes are well stained and are counted simultaneously with the platelets.

2. This method has the following advantages: (*a*) It is simple. (*b*) It is accurate. (*c*) It permits accurate recognition of individual platelets and reticulocytes and study of their morphologic aspects. (*d*) It combines in one preparation two hematologic methods: platelet counting and reticulocyte counting.

3. Normally, there is slight variation in the size and shape of the blood platelets.

4. The normal blood platelet count with this method in men ranges from 500,000 to 900,000 per cubic millimeter. The normal count in women is made uncertain by the complicating presence of the menstrual cycle. The intermenstrual range is from 400,000 to 800,000 per cubic millimeter.

5. Various procedures to check the accuracy of the method were undertaken. The experimental error is probably not greater than 70,000 per cubic millimeter when the erythrocyte count is normal and not more than 10,000 per cubic millimeter when the erythrocyte and blood platelet counts are very low.

KIDNEY WEIGHT, BODY SIZE AND RENAL FUNCTION

EATON M. MacKAY, M.D.
LA JOLLA, CALIF.

In 1916, Addis [1] introduced the determination of the ratio $\frac{\text{urine urea rate}}{\text{blood urea concentration}}$ under certain standard conditions as a measure of the amount of functioning renal tissue. The adequacy of this ratio as measured under the standard conditions for this purpose was demonstrated in a number of ways. It is a reasonable measure of renal function; [2] the variation in "experimental nephritis" was found [3] to agree well with the structural changes in the kidneys, and the compensatory renal hypertrophy as measured by the ratio coincided with the anatomic measurements.[4] Lastly, it was shown [5] that in rabbits there existed a linear relationship between the magnitude of the ratio and the weight of the kidneys, an observation since confirmed for the rat [6] and the dog.[7] Taylor, Drury and Addis [5] found that in rabbits the kidney weight varied directly in proportion to the body surface and bore a more constant relation to this figure than to any other measure of body size. This observation along with the similar finding by Stewart [8] in the dog and the more constant relation of the ratio of urea excretion to body surface than to other measurements of body size in man led Addis [9] to assume a direct relationship between kidney weight and body surface and to correct his ratio for urea excretion, when used for measuring the amount of functioning renal tissue in

---

From the Scripps Metabolic Clinic.

1. (*a*) Addis, T., and Watanabe, C. K.: J. Biol. Chem. **28**:251, 1916. (*b*) Addis, T.: J. Urol. **1**:263, 1917; (*c*) Renal Function and the Amount of Functioning Tissue, Arch. Int. Med. **30**:378 (Sept.) 1922.

2. Addis, T.: Am. J. M. Sc. **176**:624, 1928.

3. Watanabe, C. K.; Oliver, J., and Addis, T.: J. Exper. Med. **28**:359 (Sept.) 1918.

4. (*a*) Addis, T.; Myers, B. A., and Oliver, J.: The Regulation of Renal Activity, Arch. Int. Med. **34**:243 (Aug.) 1924. (*b*) Oliver, J.: The Regulation of Renal Activity, Arch. Int. Med. **34**:258 (Aug.) 1924.

5. Taylor, F. B.; Drury, D. R., and Addis, T.: Am. J. Physiol. **65**:55, 1923.

6. MacKay, E. M., and Raulston, B. O.: J. Exper. Med. **53**:109, 1931.

7. MacKay, E. M.: Am. J. Physiol. **100**:402, 1932.

8. Stewart, J. N.: Am. J. Physiol. **58**:45, 1921.

9. Addis (footnote 2). Addis, Myers and Oliver (footnote 4*a*).

patients, according to the body surface of the subject. Additional evidence in support of this practice has been offered by McIntosh, Möller and Van Slyke.[10] It has been indicated elsewhere [11] that the expected kidney weight could be predicted in man most accurately from the surface area as a measure of body size. However, the evidence for this statement was not presented, and it seems desirable to demonstrate that body surface is the best reference standard for renal weight in man.

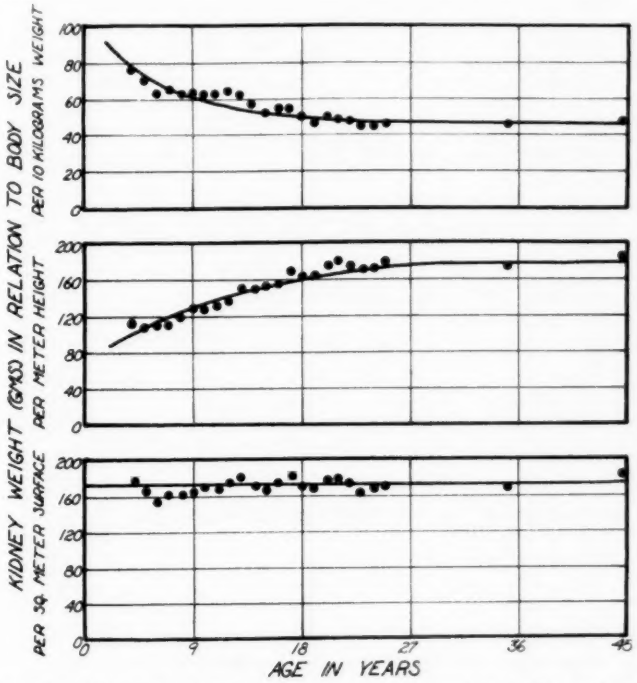


Chart 1.—Weight of kidney in relation to body surface, height and weight, plotted against age.

KIDNEY WEIGHT IN RELATION TO BODY SIZE

The figures quoted by Vierordt [12] for body weight, height and kidney weight in relation to age were used. Body surface was calculated

10. McIntosh, J. F.; Möller, E., and Van Slyke, D. D.: J. Clin. Investigation **6**:467, 1929.

11. MacKay, E. M., and MacKay, L. L.: J. Clin. Investigation **4**:127, 1927.

12. Vierordt, H.: Anatomische, physiologische und physikalische Daten und Tabellen, ed. 3, Jena, Gustav Fischer, 1906, pp. 8, 22 and 36.

by the usual formula of DuBois.[13] In chart 1, kidney weight in relation to these three measures of body size has been plotted against age. In a general way the relationship is similar to that reported for the albino rat.[14] The kidney weight in relation to body weight decreases with age and in relation to body length, as might be expected, increases with age. The relation of kidney weight to body surface is practically the same at all ages, being a direct one. This is shown even better

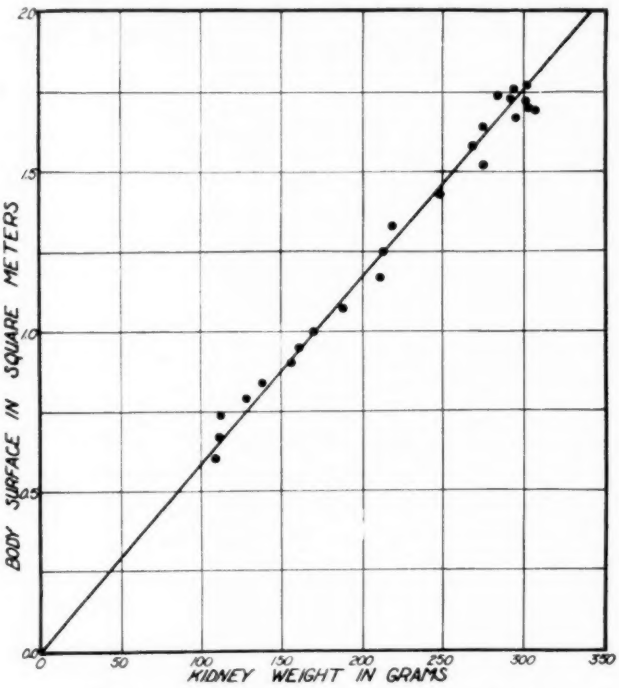


Chart 2.—Relation of body surface to kidney weight.

in chart 2. It has already been pointed out that a similar relationship exists in the rat,[14] the rabbit [5] and the dog.[8]

KIDNEY WEIGHT IN RELATION TO RENAL FUNCTION

Since kidney weight is directly proportional to body surface it follows that the Addis ratio for urea excretion will likewise be proportional to

13. DuBois, E. F.: Basal Metabolism in Health and Disease, Philadelphia, Lea & Febiger, 1927.

14. MacKay, L. L., and MacKay, E. M.: Am. J. Physiol. **83**:191, 1927.

kidney weight in man because of the direct relation that has been demonstrated [10] between body surface and the urea ratio. The data of McIntosh, Möller and Van Slyke [10] on the relation between body surface and the high volume urea ratio have been reproduced in chart 3 (*A*) and compared with some new figures (*B*) of our own on a number of children and young adults, all males. From the relationship found in chart 2, the kidney weight has been determined indirectly, and its relation to the urea ratio is shown. This is a direct one.

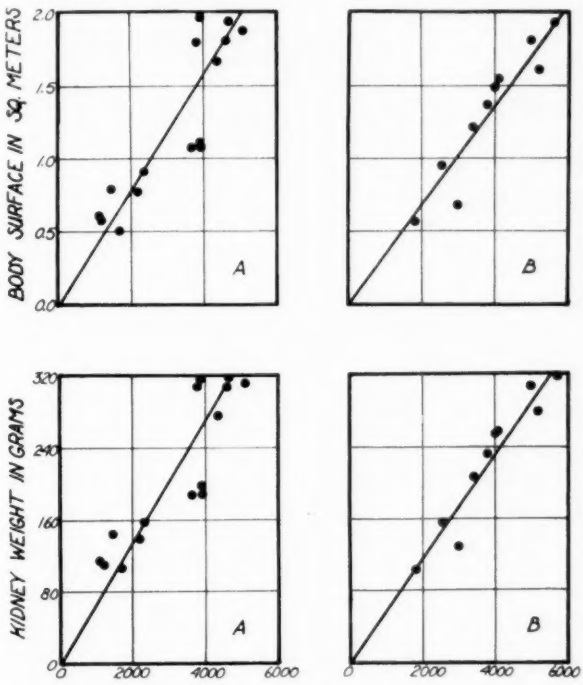


Chart 3.—Relation of kidney weight to the urea ratio. Addis ratio: $\frac{\text{urine urea rate — mg. per hour}}{\text{Blood urea concentration — mg. per 100 cc.}} \times 100$

A illustrates the data of McIntosh, Möller and Van Slyke and *B*, those of MacKay.

In chart 3 the relation between the body surface and the high volume ratio: $\frac{\text{Mg. urine urea per hr.}}{\text{Mg. urea per cc. blood}}$ is somewhat different in the two sets of observations. The average of the figures (*A*) from McIntosh, Möller and Van Slyke [10] is 2,524 cc., while the average of our figures (*B*) is 2,970 cc. per square meter of body surface. The only difference in the method of obtaining the two sets rests in the use of an ideal

weight for height in obtaining the body surface of the "*A*" group while the actual body weight was used for calculation of the "*B*" group. We have recalculated the "*A*" figures on the latter basis, but the result is essentially the same. Since the average of these two sets of figures, 2,757, so closely approximates the average of 2,770 cc. per square meter found by Addis [15] in 163 observations on 31 young adults, it seems best to retain this figure, which has been the normal standard in Addis' laboratory [16] for many years. From what is known of kidney weight in relation to body surface in animals and to sex it seems probable that the standard for the female should be lower than for the male sex. However, as McIntosh, Möller and Van Slyke [10] have noted, the difference is probably immaterial for practical purposes: in any case, data sufficient to make the distinction are not available.

SUMMARY

In man renal function as measured by the ratio $\frac{\text{urine urea rate}}{\text{blood urea concentration}}$ under certain standard conditions and kidney weight are both directly proportional to the body surface.

15. Addis (footnotes 1 *b*, 2 and 4).

16. McIntosh, Möller and Van Slyke (footnote 10). Volhard, F.: Nieren und ableitende Harnwege, Berlin, Julius Springer, 1931, p. 155.

NONTROPICAL SPRUE WITH DUODENAL INVOLVEMENT AND TETANY

ROBERT B. RADL, M.D.
AND
MADELEINE FALLON, M.D.
MINNEAPOLIS

It is easy to distinguish several indirect effects of the introduction of the liver therapy in pernicious anemia by Minot and Murphy. The differential diagnosis between pernicious anemia and other obscure and severe anemias has been sharpened. The rôle of the gastro-intestinal tract in the pathogenesis of anemias has been stressed and the nutritional factor more strongly emphasized than during the preceding ten years of high tide of interest in vitamins.

The following study of a single case is presented because of the anemia, for some time looked on as pernicious; because of the sprue-like disturbance of intestinal absorption and motility; because of the tetany resulting from this disturbance, and finally because of marked duodenal changes and their relation to the other symptoms.

REPORT OF A CASE

History.—Mr. A. J., aged 41, born in Wisconsin of Swedish extraction, is an unmarried farmer. He has never been away from the north central region of the United States. Though in general his past health has been good, he was never a robust child. There is nothing to indicate any unusual dietary mismanagement. He had pneumonia at the age of 4. At 10, his knee joints were stiff, swollen and painful, but there was no fever, he was not confined to bed, and there were no sequelae. Roughness and furrowing of the finger-nails have been noted for about seventeen years. Bilateral incomplete inguinal hernia has been noted for three years.

First Admission to Hospital (July, 1928).—The first symptom of the present illness was soreness of the tongue, which began insidiously about eight years before his admission to the hospital. It was intermittent and severe enough to cause him to select bland foods. The first gastro-intestinal symptoms occurred about six years later, when a change in the character of the stools was observed. He noted an urge to defecate about 3 or 4 a. m. The stools were larger than normal, semi-solid, normal in color but not frothy, nor was blood or mucus ever noticed. His general condition remained stationary for about a year. Then the stools gradually increased in number; there were usually two movements early each morning. Defecation did not afford the usual sensation of relief, and there was abdominal

From the Medical Service of the University Hospital and the Department of Medicine, University of Minnesota.

discomfort. The appetite remained undisturbed, but the patient felt fatigued and lost about 10 Kg. in weight. There was no nausea or vomiting. He had felt numbness and tingling of hands and feet for about four years more or less constantly.

Physical Examination (1928).—The patient did not appear acutely ill. He was poorly developed, of apparently subnormal constitution. His posture was drooping, his stature undersized, his shoulders narrow (fig. 1). He weighed 49 Kg. and was 157 cm. in height. Other recorded measurements were: crown to sym-

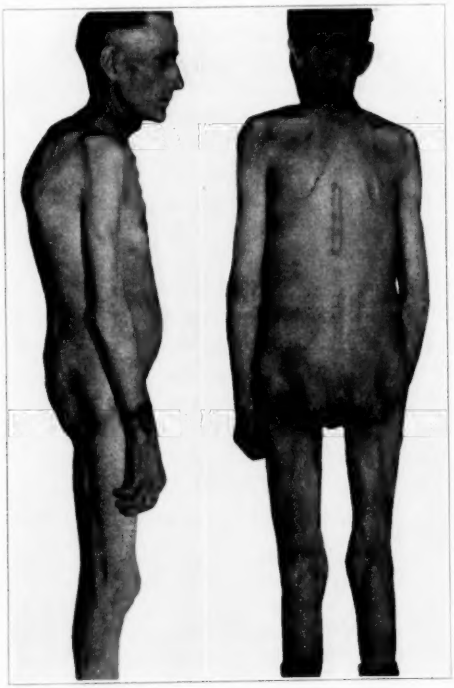


Fig. 1.—The bodily habitus of the patient.

physis, 77 cm.; symphysis to floor, 80 cm.; chest on inspiration, 82 cm., and on expiration, 76 cm.; waist, 76 cm.; iliac crests, 79 cm.; span of arms, 165 cm. There were seven upper and six lower teeth present. Onychia gryposis was present in six finger-nails. He responded sluggishly to questions and talked listlessly and slowly. The temperature was normal; the pulse rate, from 60 to 90. The skin was dry and cool; there were no areas of pigmentation. The hair was dry, but normal in distribution. The papillae of the tongue were markedly atrophic, but no aphthae or ulcerations were present. The tonsils were submerged and a few cervical nodes slightly enlarged. The thyroid gland was not palpable. The heart and lungs were normal, except for a faint, systolic murmur heard over the aortic area. The blood pressure was 100 systolic and 70 diastolic. No other

abnormalities were noted, except evidence of bilateral inguinal hernias. All reflexes were normal. The joint position sense and vibration sense were normal.

Laboratory Data.—The result of a Wassermann test of the blood was negative. The urine was normal on various occasions. An Ewald meal showed no free hydrochloric acid present. The stools were light yellow and contained undigested particles of food. No blood, ova or parasites were found. The results of roentgen examination of the stomach and duodenum are illustrated in figure *2*; they are discussed in later paragraphs. The blood count (Aug. 1, 1928) revealed 2,870,000 erythrocytes and 5,000 leukocytes; the hemoglobin content was 64 per cent (Sahli). The color index was 1.14. There were moderate poikilocytosis and marked anisocytosis with many macrocytes. After liver therapy (*2* capsules of liver extract four times daily) the following improvement occurred: On October *22* to *28*, the red cell count was 5,700,000; the hemoglobin content, 85 per cent.

Clinical Course.—The patient felt better as a result of the administration of liver extract. He gained 5 Kg. in weight; the blood improved, but the bulky stools persisted. He was discharged on Sept. 13, 1928, with a diagnosis of pernicious anemia without neurologic changes.

Second Admission (Feb. 17, 1930).—The patient had continued to feel well. He claimed to have taken one-half pound (227 Gm.) of liver daily for about a year. In September, 1929, the taking of liver was discontinued because of distress, fulness or pressure in the epigastrium. The epigastric pain was partially relieved by eating. The gastric symptoms became worse and were aggravated by fried foods or coarse vegetables. Nausea and vomiting had occurred intermittently following December, 1929. Vomiting and belching relieved the distress. The early morning bowel movements persisted, the number of stools varying from two to five. They were of the same character as described in the previous paragraphs.

Physical Examination.—No changes were revealed except that a vague mass was palpated 3 cm. below the right costal margin. It was not tender or movable. The gastric secretion was tested after 0.5 mg. of histamine had been given; free hydrochloric acid was present with Günzburg's test after forty minutes, but not after twenty or sixty minutes. Roentgen examination (Feb. 21, 1930, fig. *2 b*) showed a dilated stomach and duodenal stenosis (see later paragraphs). The blood pressure was 94 systolic and 64 diastolic. The response to medical treatment for peptic ulcer was poor, and on March 10, 1930, a typical posterior gastroenterostomy was performed with the anastomosis made transverse along the greater curvature (Dr. L. W. Tasche). The findings at operation were described as follows: A large duodenal ulcer extended over almost the entire first portion of the duodenum, narrowing it very much. The second portion of the duodenum seemed normal. The lesser peritoneal sac showed signs of recent inflammation by adhesions.

Convalescence was uneventful. The patient was discharged on March 31, 1930, with the diagnosis recorded as duodenal stenosis with partial obstruction.

Third Admission (June 17, 1930).—Although the patient had gained slightly in weight he had not improved much. There had been no vomiting, but the loose bowel movements persisted. In May he had an attack of severe, painful tetanic contracture and flexion of the forearms, hands and legs below the knees. The fingers were flexed over the adducted thumb. Similar attacks recurred at intervals of about five days lasting from thirty minutes to three hours. Slight edema of the legs was noted. The edema was unaffected by posture.

Physical Examination.—There were no new findings, except that Chvostek's and Trousseau's signs were present. The abdominal and deep reflexes were absent,

except during attacks of tetany, when they were present and increased over the normal in intensity. There was slight pitting edema of the lower legs. No studies of the blood calcium were carried out on this admission. By the administration of calcium lactate and parathyroid extract the attacks of tetany were controlled. The patient's weight increased from 38 to 41 Kg., while his edema diminished. His appetite was fair; the addition of dilute hydrochloric acid had no apparent effect. He was discharged on Aug. 6, 1930.

Fourth Admission (Sept. 17, 1930).—About two weeks before the fourth admission, tetany recurred. Attacks came on daily and lasted from one-half to one hour. The edema of the legs increased markedly, especially that in the left leg. There was considerable abdominal distention. The blood pressure was from 96 to 100 systolic and from 69 to 70 diastolic. The stools were unchanged in character, but had increased in number to five or more daily. The stool was bulky, semisolid and light yellow, weighing on the average 16 ounces (475 Gm.); they were never frothy. When the stools were frequent, they tended to be watery. Microscopic examination showed undigested particles of food and globules of fat. Stercobilin was present; blood was never found.

Analysis of the gastric juice showed hydrochloric acid to be present on one occasion after injection of histamine and absent on another. The total acidity varied between 14 and 36; the total chlorides in the first and the last tests with histamine were as given in the table.

Total Chlorides of Gastric Juice, Mg. per Hundred Cubic Centimeters

	Before Gastro-Enterostomy (2/19/30)	After Gastro-Enterostomy (12/16/30)
Fasting	228	327
20 min.	304	246
40 min.	339	327
60 min.	382	331

Two separate determinations of the basal metabolic rate, when no tetanic cramps were present, gave values of —19 and —21. An earlier determination, disturbed by continuous cramps, had given a value of +30. A dextrose tolerance test gave a normal curve.

The lowest value determined for the serum calcium was 5 mg. per hundred cubic centimeters. With sufficient calcium lactate (12 Gm. daily) and parathormone (10 to 30 units daily) given to keep the patient free from tetany, the serum calcium was usually found between 7 and 9.8 mg. The inorganic serum phosphorus was 4.2 mg., while the calcium was 7 mg.

The plasma proteins (May 4, 1931) were 6.48 per cent total protein, 3.16 per cent albumin and 3.32 per cent total globulin. The globulin fraction was composed as follows: fibrinogen, 0.72 per cent; euglobulin, 0.56 per cent; pseudoglobulin I, 1.48 per cent, and pseudoglobulin II, 0.56 per cent. The blood nonprotein nitrogen was 35 mg. per hundred cubic centimeters.

Roentgen Observations.—The roentgen studies carried out during the course of our observations are summarized by Dr. Leo Rigler as follows:

The first examination (July 13, 1928) revealed some hyperperistalsis, and at times, spasticity of the pylorus. Some relaxation of the pylorus also took place. The duodenal bulb was rather small, but normal otherwise. There was a marked narrowing of the second, and to some extent of the third, portion of the duodenum, suggesting some type of stenosis. A diverticulum of the jejunum was also made out.

The next examination (Jan. 31, 1930) showed a marked change in the appearance. The stomach was distinctly dilated, and there was marked hyperperistalsis. Marked stenosis of the pylorus was present. The duodenal bulb was extremely small and irregular, and there was marked stenosis of the second and third portions of the duodenum, which had increased considerably since the last examination. The appearance suggested a rather extensive ulcerated process of the duodenum with marked periduodenal adhesions. The diverticulum previously reported was again shown. The third examination (Feb. 21, 1930) showed about the same changes. About 80 per cent retention in the stomach was present after six hours. The next examination (March 5, 1930) showed such extreme retention in the stomach that it was impossible to visualize the small bowel.

The next examination (March 24, 1930) showed a well functioning gastro-enterostomy. There was still a small trace of barium passing through the pylorus

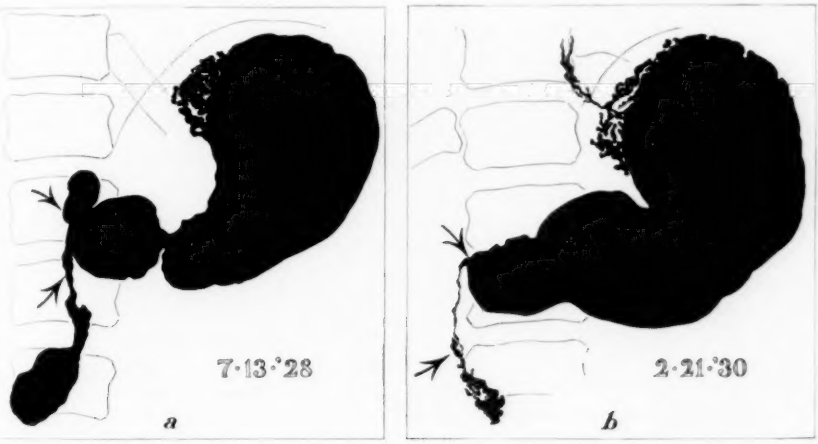


Fig. 2.—Drawings showing roentgenographic appearance of stomach and duodenum (*a*) July 13, 1928, and (*b*) Feb. 21, 1930.

and duodenum, but these were difficult to visualize. The stomach was reduced in size. The next examination (June 18, 1930) again showed the gastro-enterostomy functioning well, with no other evidence of a pathologic condition.

The final examination (Nov. 19, 1930) showed the gastro-enterostomy functioning fairly well, but much more barium passing through the pylorus. The stenosis of the duodenum previously reported was again shown, and the third portion of the duodenum showed some dilatation. There was also some regurgitation from the third portion of the duodenum into the pylorus.

Repeated examinations of the colon were made. These showed extreme redundancy of the colon, but no other evidence of a pathologic condition. Examination of the pelvis, skull and femur on Oct. 24, 1930, showed some decalcification of the pelvis, but this was slight, and the clinical significance of it is questionable. Examination of the gallbladder on Feb. 24, 1930, was made. This was not satisfactory; no shadow of the gallbladder could be made out, suggesting that it was not functioning well.

The conclusions were: (1) dilated stomach showing hyperperistalsis; (2) stenosis of the pylorus and of the first, second and third portions of the duodenum of extreme degree, with gastric retention; (3) diverticulum of the jejunum; (4) gastro-enterostomy, functioning well; (5) redundancy of the colon; (6) questionable involvement of the gallbladder, and (7) slight decalcification of the pelvis.

The Blood.—The blood never presented a convincing morphologic picture of pernicious anemia. The red cells at first showed moderate anisocytosis with macrocytes, normocytes and microcytes. The macrocytes were hyperchromatic, and some of the normocytes and microcytes were hypochromatic. In the blood smear, the color index appeared to be only slightly above 1. In smears taken on later admissions, there was a predominance of macrocytes, so that the color index appeared to be increased considerably, and a normal amount of polychromatophilia was present. There was always from a moderate to a slight amount of poikilocytosis. Occasionally basophilic stippling was seen. No nucleated red cells were present.

The neutrophils were normal in size and percentage. The nuclei were moderately shifted to the right (from 3 to 8 lobes), and the granulation was uneven in size and tended to be basophilic (toxic granulation). An occasional neutrophil was slightly larger than normal, but none of them had the large, even pink granulation seen in the neutrophils of pernicious anemia.

The lymphocytes were not increased in percentage. They were chiefly the large lymphocytes with large azure granules. The monocytes were reduced in percentage, being almost entirely absent in the early smears, but reaching a low normal percentage in the later smears. They presented toxic changes. The platelets were reduced in number in the early smears, but later appeared to be about normal in number and larger in size.

The blood picture remained fairly constant throughout, with the exception of the increase in the size of the red cells, the slight increase in the amount of polychromatophilia, and the variation in the number and size of the platelets.

Since the blood smears contained the toxic type of neutrophils rather than the neutrophils of pernicious anemia, and since the percentage of neutrophils was normal, we were not dealing with a true picture of pernicious anemia.

Response to Therapy.—On Aug. 1, 1928, the patient's blood had a hemoglobin content of 64 per cent (Sahli); the red cell count was 2,870,000, and the white cell count, 4,950. After forty-three days on liver therapy (a concentrated liver preparation, 8 capsules daily) the hemoglobin content was 91 per cent; the red cell count, 5,650,000. He was discharged and told to eat one-half pound of raw liver daily. He continued taking liver, alternating raw liver and liver extract until September, 1929, when he began to take it irregularly. During this year on liver, his hemoglobin remained at a high level, 79 to 85 per cent, but the red cell count ranged from 3,400,000 to 4,400,000.

Following the second admission, he was given raw linseed oil (5 cc. three times daily) for eleven days. There was a slight increase in the hemoglobin, from 89 to 99 per cent, and a slight change in the red cell count, from 3,410,000 to 3,980,000. The reticulocyte count ranged from 0 to 1.6 per cent.

Following the gastro-enterostomy there was no treatment for four months. Then over a period of thirty-nine days he was given calcium lactate and parathormone. At the end of this period, the hemoglobin content was 63 per cent, the red cell count 3,050,000 and the white cell count 4,200.

On the fourth admission, raw linseed oil was tried again in larger dosage (45 cc. three times daily). The hemoglobin increased from 73 to 80 per cent. The

red cell count rose from 2,880,000 to 3,390,000, but both dropped again to the original levels. The reticulocyte count varied from 1 to 4.2 per cent.

Iron ammonium citrate (6 Gm. daily) was then given with a high caloric, high vitamin diet over a period of twenty-two days, with no appreciable change in the hemoglobin, red cell count or reticulocyte count.

A diet high in protein but low in salt and sugar-free was tried for two weeks. Although there was no significant change in the percentage of hemoglobin or in the number of red cells, the reticulocyte count rose to 4.2 per cent.

A general diet with extra fat, butter, cream and milk was given for two weeks. The reticulocyte count ranged from 2 to 2.5 per cent.

Then liver extract no. 343 (8 vials daily) was given for eighteen days with a diet high in protein, but low in sugar and fat. The hemoglobin increased from 82 to 90 per cent, but fell again to 74 per cent. The red cell count increased from 2,820,000 to 3,480,000, but fell to 2,990,000. The reticulocyte count varied from 2 to 3.6 per cent.

The same diet was maintained, but 150 Gm. of raw calves' liver was substituted for the liver extract. The hemoglobin gradually rose to 90 per cent, and the red cell count reached 4,630,000. The reticulocyte count varied from 1.4 to 3.4 per cent.

COMMENT

The first symptom was sore tongue, present eight years prior to the first observation. When the patient was first seen, the anemia was not sufficiently marked to produce a typical morphologic picture of pernicious anemia, while the improvement following medication with liver extract was compatible with such a diagnosis. The patient's appearance, one of general debility (fig. 1), was much against pernicious anemia. There were reasons why adequate consideration was not given to the roentgen evidence of a pathologic duodenum: There were not the usual subjective symptoms of duodenal ulcer; neither were the films characteristic of such a condition; the abnormalities of the stools of two years' duration seemed compatible with pernicious anemia, as was the achlorhydria in the Ewald meal, the latter compatible also with duodenal ulcer. The absence of spinal cord symptoms was not incompatible with pernicious anemia of moderate severity.

The rapid development of the duodenal lesion into a stenosis of considerable length dominated the second hospitalization, while the anemia remained much the same as when the patient was first seen. Again it is noted that the pain and discomfort associated with the duodenal changes were slight up to the time when symptoms of retention developed. Symptoms of earlier perforation could not be elicited. Though there was no indication for opening the duodenum during the operation, the observations made it possible with moderate certainty to rule out malignant tumor, tuberculosis and syphilis.

With the facilities for adequate nutrition mechanically improved by a well functioning gastro-enterostomy, the patient nevertheless grew worse, a definite condition of deficiency being signaled by the début

of tetany. Interest in this condition lately has been greatly intensified, and a variety of causes have been described. Its occurrence in sprue is often referred to, while mention of its occurrence in cases of pyloric stenosis with dilatation of the stomach and copious or prolonged vomiting is becoming very rare. Association of its occurrence with pancreatic disease recently has been placed in abeyance by British writers (Linder and Harris [1]); Berglund, however, in a case seen in consultation, saw severe tetany in combination with the typical symptoms of pancreatic insufficiency, the latter having developed acutely immediately after an operation for perforated duodenal ulcer; in the course of the operation, the pancreatic duct had been caught and closed by a suture. During his sojourn in Peiping, Berglund also observed tetany in an otherwise normal young Chinese mother, in whom the drain on the calcium supply produced by lactation was sufficient to precipitate tetany. For all these forms it appears certain "that the tetany has its basis in a deficiency of calcium" (Linder and Harris [1]), of which a low level of the serum calcium is an indication.

On the basis of the roentgenogram (fig. 2 *b*) of our patient, one might consider the possibility of secondary occlusion of the pancreatic ducts. The abnormality of the stools is hardly pronounced enough to correspond to the typical syndrome of pancreatic exclusion; the bulky and frequent stools were present a long time before the duodenal condition was marked, and the aggravation of the condition has been very gradual. An interpretation nearer at hand is that of nontropical sprue. The number of such cases that have been reported both from Europe and America is sufficient to have made the condition well recognized; some cases closely correspond to the picture of tropical sprue; others are less well defined. The following symptoms and signs may be said to constitute a typical picture of the moderately advanced case: dyspepsia, flatulence, capricious appetite, constipation alternating with voluminous liquid or semiliquid stools, which are often foamy and light yellow, glossitis or stomatitis, anemia of pernicious type, muscle cramps, loss of weight, low blood pressure and mental depression. Secondary symptoms may be present, such as edema of the extremities and tetany with low blood calcium.

The following detailed observations on our patient deserve brief comment: Monilia pinoyi was isolated from the stools and identified by Dr. A. T. Henrici of the department of bacteriology. In regard to etiology, little significance is placed on this finding. It is commonly believed that the incomplete digestion and the fermentation merely provide favorable conditions for its growth.

1. Linder, G., and Harris, C. F.: Quart. J. Med. **23**:195, 1930.

The general debility is expressed in many ways besides in the exterior of the patient. That there are numerous possibilities for faulty intestinal absorption does not have to be emphasized. The patient for more than a year has had recurrent superficial ulcers of the cornea. Xerophthalmia is not present; neither have the ulcers shown the same dangerous tendency to perforate as did the instances of keratomalacia frequently observed by Pillat in Peiping among adult Chinese suffering from a deficiency of vitamin A. There is no cataract, which frequently is present in parathyroid tetany.

The basal metabolic rate is below the range of normal variability. Of interest is the degree of its elevation, from — 20 to + 30 during the active state of tetany.

Contrasted against the low metabolic rate and the wasted condition of the musculature are the concentrations of the plasma proteins, which are well within normal variability. The fibrinogen is above the normal range and a little more than twice the normal mean. Serum calcium is pathologically low. There is no creatinuria.

The ability to secrete hydrochloric acid is not completely lost, at least not all the time; the secretion, when produced, is minimal. The concentration of total chlorides is below the normal mean as established by Berglund, Johnson and Chang, but well within normal variability; the values fall just within — 1, the standard deviation for their mean. The total chloride values in pernicious anemia the same investigators usually found to be lower than in this patient.

Of special tests for pancreatic secretion, only the digestion of cell nuclei, according to Adolf Schmidt, was carried out. Without vouching for the specificity of the procedure as a test for pancreatic function, we may say that the result was clearcut: Of five small gauze parcels containing sweetbread given to the patient, four were recovered showing the nuclear structures fully preserved, while from the interns who took the test simultaneously only the gauze was recovered. A dextrose tolerance test gave a normal blood sugar curve. Too much significance should not be attached to this finding. The rate of dextrose absorption may have been reduced and may thus have influenced the curve.

The blood in 1928 responded promptly to liver extract; in 1931, poorly, but better to raw liver than to liver extract. Considering the amazing difference recently demonstrated by the Boston group in the amount of active principle needed by mouth and when parenterally administered, the poor response in our patient was thought to be due possibly to failure of absorption. Intramuscular administration of liver extract was therefore tried (10 cc. of extract no. 343 for eight days), with no response.

Our observations clearly indicate that the anemia responded to raw liver and liver extract but not to iron or to linseed oil.

The general condition has, on the whole, been resistant to different forms of therapy; there has been no improvement similar to that observed by Porter and Rucker [2] after liver extract or by Linder and Harris [1] after restriction of the fat intake. Large doses of viosterol gave no definite improvement. The tetany has not been controlled by diet; large doses of calcium lactate and parathormone are necessary over and over again.[3]

SUMMARY

A condition that deserves the diagnosis nontropical sprue has been described, presenting, besides bulky fatty stools, anemia, duodenal stenosis and tetany. Constitutional inferiority is considered a factor of pathogenic importance. The response to different therapeutic procedures was unsatisfactory. Detailed chemical and hematologic studies are reported.

---

2. Porter, W. B., and Rucker, J. E.: Am. J. M. Sc. **179**:310, 1930.

3. Our interest being focused on the early diagnosis of nontropical sprue and the possible justification of speaking of mild and abortive forms, we wish to mention a patient under observation at the present time.

A. V. is a white man, aged 33, born in this country. The history is essentially one of having large and frequent stools intermittently for the past eleven months, sore tongue and attacks highly suggestive of tetany. A diet high in carbohydrate produces stools typical of sprue, and the condition is easily checked by a diet low in carbohydrate and low in fat. There is a normal hydrochloric acid content of the gastric juice; there is no anemia. The result of the sweetbread test is normal. But for our interest in sprue and the history of sore tongue and tetany, the condition probably would have been labeled fermentation dyspepsia as conceived by Adolf Schmidt.

EXPERIMENTAL STUDIES IN GASTRIC PHYSIOLOGY

EVALUATION OF THE RÔLE OF DUODENAL REGURGITATION IN THE CONTROL OF GASTRIC ACIDITY IN MAN (BOLDYREFF THEORY)

HARRY SHAY, M.D.

ALBERT B. KATZ, M.D.

AND

EUGENE M. SCHLOSS, M.D.

PHILADELPHIA

Since Heidenhain's [1] observation that the pure gastric juice in the normal dog was secreted at an acidity of 0.5 to 0.6 per cent hydrochloric acid, and particularly since its confirmation in human beings by Carlson,[2] physiologists have puzzled over the mechanism involved in the reduction of this high acidity to the one ordinarily seen in gastric contents. In general, the many theories evolved to explain the reduction concern themselves with one or the other of two mechanisms: (1) neutralization and (2) dilution. Babkin [3] recently gave an excellent résumé of this subject. It is our intention to inquire into only one of these theories, the one that has doubtless enjoyed the greatest popularity. This is the theory developed by Boldyreff and styled by him "the self-regulation of gastric acidity." Briefly, it contends that the small intestine cannot tolerate an acid stronger than from 0.1 to 0.15 per cent hydrochloric acid. In order to render the gastric acid acceptable to the small intestine, it must be reduced from an approximate concentration of 0.5 to 0.15 per cent. Boldyreff [4] stated that the strong acid, passing from the stomach into the intestine, provokes an abundant secretion, chiefly of pancreatic juice. If this is insufficient,

From the Gastro-Intestinal Clinic of the Jewish Hospital, Philadelphia.

Read before the Physiological Society, University of Pennsylvania, Jan. 11, 1932.

Presented in abstract before the Section of Medicine, College of Physicians, Philadelphia, Dec. 28, 1931.

1. Heidenhain, R., quoted by Boldyreff: Quart. J. Exper. Physiol. **8**:1, 1914.

2. Carlson, A. J.: The Control of Hunger in Health and Disease, Chicago, University of Chicago Press, 1916, p. 255.

3. Babkin, B. P.: Physiological Factors Determining Acidity of Gastric Juice and of Gastric Contents, Canad. M. A. J. **17**:36, 1927.

4. Boldyreff, W.: The Self Regulation of the Acidity of the Gastric Contents and the Real Acidity of the Gastric Juice, Quart. J. Exper. Physiol. **8**:1, 1914.

bile and intestinal juices are also secreted. He further contends that the entrance of the strong gastric acid into the duodenum, through its irritant action on the duodenal mucosa, causes antiperistalsis in the duodenum. This, in turn, drives the alkaline secretion of the intestine into the stomach until there is accumulated therein a quantity of alkaline material sufficient to lower the acidity of the gastric contents to the usual level of about 0.15 per cent hydrochloric acid.

Since Beaumont's [5] celebrated work on the physiology of digestion, regurgitation of intestinal contents into the stomach had been regarded more or less as a pathologic phenomenon. The work of Boldyreff, however, seemed to give a definite and an important place to such regurgitation in the normal process of gastric digestion. Boldyreff's theory has the happy faculty of appealing to the popular imagination, as it can be made to explain so readily many things concerned with gastric acidity and the pyloric mechanism. A careful survey of the literature, however, fails to reveal convincing evidence in its favor. On the other hand, it appears that definite evidence from human and, especially, from animal experimentation which tends to refute the theory has been either missed or ignored. Our most recent works on physiology still present this theory of the control of gastric acidity as an established fact.

The results of our experiments, on human beings, would indicate that the occurrence of duodenal regurgitation is not a purposeful physiologic phenomenon, but merely incidental to the resistance shown by the duodenum to the entrance of irritants; if the irritant is strong enough, its effect is reflected in the action of the pylorus. Considerable experimental literature is available regarding this theory; however, since it will be conducive to clearness to set forth the results of these investigations in connection with our own work, their consideration will be deferred until later. The results of our experiments, on human beings, fortified by those obtained by others on dogs, certainly seem to warrant a skeptical attitude regarding the efficacy of duodenal regurgitation in the control of gastric acidity.

EXPERIMENTAL METHOD

In 1922, Bolton and Goodhart stated that up to that time the views on duodenal regurgitation into the digesting stomach of the human subject were chiefly matters of surmise. From the evidence we were able to gather it appears that the same holds true for the present. Baird, Campbell and Hern [6] felt that the final solution of this problem in man

5. Beaumont, W.: Physiology of Digestion, ed. 2, Burlington, Vt., Chauncey Goodrich, 1847.

6. Baird, M. M.; Campbell, J. M. H., and Hern, J. R. B.: Importance of Estimating Chlorides in Fractional Test Meal Samples, and Some Experiments with Duodenal Tube, Guy's Hosp. Rep. **74**:23 (Jan.) 1924.

depended on finding a substance that would answer certain criteria; these were that it could be readily estimated, should not be absorbed in the stomach, should not affect the result of a test meal and should not be present normally in the secretions of the upper alimentary tract. We felt that in bromsulphalein we had such a substance. This dye, introduced and widely used for testing liver function, may be injected intravenously. From the blood stream, it is removed almost quantitatively by the liver (a very small quantity being normally taken out by the kidneys) and is excreted in the bile, by means of which it reaches the second portion of the duodenum. Its ready determinability, plus its other characteristics, made it an ideal substance for testing duodenal regurgitation.

In all of the experiments, 5 mg. per kilogram of body weight were used. For obvious reasons, each patient used in the study had first to be standardized with regard to (1) removal of the dye by the liver, (2) regularity of appearance time of the dye in the duodenum, (3) concentration of the dye in the duodenum during twenty minute periods for two hours after appearance of the dye, (4) elimination of dye by the kidneys during the test period. To accomplish this, each patient received the calculated dose of dye intravenously at weekly intervals for three weeks, after first intubating the duodenum with a Rehfuss tube. At each session, the blood retention at the end of one-half hour was noted, appearance time of the dye in the duodenum determined, quantitative estimation of the concentration of the dye in twenty minute specimens of duodenal contents was made over a period of two hours after the appearance of the dye in the duodenum, and quantitative estimation made of the dye excreted in the urine during the test period.

It may be of interest to interpolate at this point an observation that may concern the action of bromsulphalein on the liver cells. While studying the appearance time of the dye in the duodenum, it was noted that with the evidence of the dye in the draining duodenal contents the flow from the tube became more regular and the rate of flow definitely increased. It appeared as if the dye had stimulated bile flow. This action may help to explain some of the instances of marked symptomatic relief experienced by patients at times after a bromsulphalein test is performed. One is inclined frequently to attribute such results to the psychic effect of an injection, but such results have been noted in patients in whom it has been difficult to accept this explanation for the very definite effects noted.

The duodenal appearance time in each subject varied but slightly on repeated examinations. The greatest variation in the group studied was two minutes. While the concentration of dye in the duodenum varied, it was only of importance to note that at all times during the two hour test periods there were very high concentrations of dye in the

duodenal contents. The blood retentions varied within 10 per cent limits, and the urinary excretion of the dye was negligible both in amount and variation. The blood determination was made by the regular technic, using the usual bromsulphalein standards. The same method was applied to the duodenal contents and urinary determinations, except that 1 cc. of the duodenal contents, after alkalinization, was diluted to 100 cc. with water, and the readings then made in the usual way. This was necessary because of the very high concentrations of the dye in the duodenal contents during the two hours of the test period.

After standardization, the patients were studied as follows: Gastric intubation was done after a twelve hour fast. The fasting stomach was completely emptied in all positions. The calculated amount of dye was injected and at the duodenal appearance time of the dye, previously determined, the stomach was again completely emptied. Four minutes after the appearance (allowing for the foregoing variation), the test meal was administered by mouth. Within five minutes after its ingestion, the stomach was again emptied completely. The amount recovered was noted, gross description made, the material withdrawn thoroughly mixed, and all but 15 cc. returned by gravity through the tube to the stomach. This procedure was repeated at twenty minute intervals for two hours, care being taken that the tube was kept at the same toothmark throughout the studies. Each specimen retained was then titrated for free and total acidity and a determination of its dye content, when present, made after alkalinization with 20 per cent sodium hydroxide by the regular bromsulphalein technic. At successive weekly intervals, the following test meals were used: 200 cc. portions of tap water at room temperature, hydrochloric acid in strengths ranging around 0.2 and 0.5 per cent, sodium bicarbonate of 1 and 5 per cent. Water was first used, since it was desirable to see what response the subject would show to water, as it was to form the solvent for our other test substances. In all, more than sixty determinations were so made. Cases were selected to include the entire range of gastric acidity, from achylia gastrica to hyperchlorhydria. Knowing the approximate concentration of dye in the duodenum, measuring the amount of gastric contents and determining the concentration of dye, when present, in this mixed specimen, enabled us to form a definite idea of the amount of regurgitation that must have taken place. We do admit that duodenal regurgitation into the stomach can and doubtlessly frequently does take place, but from our experiments, we cannot ascribe any importance to this mechanism in the regulation of gastric acidity. We believe that regurgitation may represent either one of two phenomena. First, it may occur incidental to the process in which the duodenum attempts to reject irritants regardless of their nature (acid, alkalis, other irritants). This regurgitation, we believe, is part of a reversal phenomenon of the upper

gastro-intestinal tract which has its most sensitive mechanism in the duodenum and its least sensitive in the mouth. An irritant taken into the mouth, if sufficiently strong, sets up stimuli which eventuate in expectoration. Should the irritant get past the mouth and be too irritating for the stomach, nausea and vomiting result. A still less irritating substance, not producing vomiting, may be too strong for the more sensitive duodenum, and as a result, duodenal regurgitation occurs. Irritants beyond this point are usually rushed analward, as we shall show in another publication.[7] Of considerable interest is the fact that frequently the more irritating the test meal, the less marked was the regurgitation. This will be shown elsewhere [8] to be due to a marked pylorospasm and anal spasm. The second phenomenon frequently responsible for regurgitation is achlorhydria. In these cases, it is due to an absence of any real pyloric control. The water meal in these instances will frequently produce more marked regurgitation than the more irritating meals which, through their very irritant action, establish some semblance of normal pyloric action and gastric emptying. This observation will also help to indicate why Iwanow [9] was misled in his interpretation of the frequent and marked regurgitation in achlorhydria and hypochlorhydria. He inferred that the regurgitation may be an important part of the mechanism in the production of the decreased acidity. Our experiments, on the other hand, indicate that the regurgitation is evidence of a poorly controlled pyloric mechanism, and is the result rather than the cause of achlorhydria.

Similarly, our roentgenographic experiments do not warrant Cathcart's [10] statement that, as a general rule, the stronger the acid introduced into the fundus of the stomach, the earlier the regurgitation takes place.

The five cases cited in table 1 are merely representative of the group studied. The figures in this table certainly present several striking facts. There can be little doubt that on the introduction of a strong acid into the stomach, some adjusting mechanism that is very rapid in action sets in; further, that this mechanism certainly cannot be concerned with duodenal regurgitation. This is so, both because of the small quantitative increase in the gastric contents and, particularly, because of the practical absence of regurgitated dye, a substance which was known to be present in the duodenum in such high concentration at the time. Column A represents the approximate amount of pancreatic

7. Gershon-Cohen, J., and Shay, H.: Control of the Pylorus, to be published.

8. Shay, H., and Gershon-Cohen, J.: Gastric Reaction and Gastric Emptying, to be published.

9. Iwanow, W.: Ueber die Regurgitation des Duodenuminhaltes in den nüchternen Magen, Arch. f. Verdauungsk. **38**:223, 1926.

10. Cathcart, E. P.: Reflux from Intestine to Stomach, J. Physiol. **42**:433, 1911.

juice necessary to produce the difference between T_1 and T_2, an amount far beyond the power of any pancreas to secrete.[11] As a matter of fact, if we might anticipate some of our future report,[8] we were able to demonstrate in our roentgenographic studies very marked pylorospasm and frequently antral spasm produced by acid similar in strength and amount to that used in these experiments; such degrees of spasm readily explain the failure of dye regurgitation above noted. *Verdünnungsaft* alone could not account for the marked drop in acidity because of the slight changes in volume, nor could pyloric secretion alone be responsible for such reductions. Pyloric secretion, though known for a long time to be alkaline (Klemensiewicz),[12] is only faintly so, and of a low rate of secretion (Ivy,[13a] Takata[13b]). It would appear, therefore, that this rapidly adjusting mechanism may likely be one of absorption, since

TABLE 1.—*Five Minute Loss in Acidity of Gastric Contents with the Actual Amount (DR) of Duodenal Regurgitation and the Theoretical Amount (A) of Regurgitated Pancreatic Juice that Would Be Necessary for Such Reduction* *

Name	T_1	T_2	A (Cc.)	G C	D (per Cent)	D D (per Cent)	D R (Cc.)
E. R.	140	100	103	230	0	1,000	0
A. C.	150	120	77	150	20.0	4,500	1.2
M. M.	140	102	97	180	0	5,000	0
G. H.	135	80	142	260	35.0	5,000	1.8
H. B.	151	100	182	240	2.5	4,000	0.15

* The test meal was given in 200 cc. quantities. T_1 indicates the titration of the test meal in terms of tenth-normal hydrochloric acid; T_2, the titration of free hydrochloric acid (tenth-normal) in the total gastric contents, removed within five minutes after ingestion of the test meal; A, the approximate amount of 0.65 per cent sodium carbonate necessary to produce the reduction from T_1 to T_2; G C, the amount of gastric contents removed from the stomach within five minutes after the ingestion of 200 cc. of the test meal; D, the percentage of dye in gastric contents in G C; D D, the percentage of dye in duodenal contents at same time as D, this reading having been previously determined; D R, the amount of regurgitation of D D necessary to produce concentration of dye found in D.

Note.—Regular bromsulphalein standards were used to determine concentration of dye.

this could account both for the rapid drop in acidity and the slight changes in volume.

We were entirely unable to correlate the amount of duodenal regurgitation as measured by the concentration of dye in the gastric contents with the degree or change of gastric acidity. As a matter of fact, the greatest amount of dye regurgitated during all the experiments yielded a reading of 360 per cent, and occurred during the course of a plain water meal in a case of true achylia gastrica (table 2). Furthermore, so far as the Boldyreff theory is concerned, it is paradoxical that the

11. Ivy, A. C.: Personal communication.

12. Klemensiewicz, R.: Ueber den Succus pyloricus, Sitzungsb. d. k. Akad. d. Wissensch., Wien **71**:221, 1875.

13. (*a*) Ivy, A. C.: Studies on the Secretion of the Pyloric End of the Stomach, Am. J. Physiol. **49**:142, 1919; (*b*) Takata, M.: Studies in the Gastric Juice: IV. On the Pyloric Juice, J. Biochem. **2**:33, 1923.

bicarbonate test meals often showed greater evidence of regurgitation than did the acid meals (table 3). These phenomena are readily explained if the rôle of the pyloric sphincter is kept in mind as previously indicated. In addition, it is to be noted that the acid meals around 0.2 per cent were apt to produce more marked evidence of regurgitation than the meals ranging around 0.5 per cent (table 2). This also is to be expected in the light of our roentgenographic experi-

TABLE 2.—*Comparison of Duodenal Regurgitation Resulting in the Same Patient from a Water, a Strong Acid and a Weak Acid Meal, Respectively* *

Time, Min.	200 Cc. H_2O				200 Cc. 0.547% HCl				200 Cc. 0.2% HCl			
	F A	T A	G C (Cc.)	Dye, per Cent	F A	T A	G C (Cc.)	Dye, per Cent	F A	T A	G C (Cc.)	Dye, per Cent
F R	0	10	..	..	0	10	..	..	0	5	..	..
5	0	5	162	90	120	130	150	20	45	50	195	20
20	0	5	12	60	100	105	95	50	30	35	65	60
40	0	5	15	20	70	75	45	40	5	15	15	60
60	0	5	20	40	55	60	30	30	0	10	4	100
80	0	5	18	55	35	45	5	30	0	10	8	180
100	0	10	20	360	10	20	5	20	0	5	7	50
120	0	5	15	150	——— Empty ———				0	5	5	100

* F A indicates free hydrochloric acid; T A, total acidity; G C, amount gastric contents; F R, fasting residuum. The data illustrate the much higher degree of regurgitation with the water meal in a case of achylia gastrica. Also note the greater degree of regurgitation with the weaker acid meal as compared to the stronger acid.

TABLE 3.—*Comparison of Duodenal Regurgitation Resulting in the Same Patient from a Relatively Strong Acid and a Relatively Strong Alkali Meal* *

Time, Min.	200 Cc. 0.511% HCl				200 Cc. 3% $NaHCO_3$			
	F A	T A	G C (Cc.)	Dye, per Cent	F A	T A	G C (Cc.)	Dye, per Cent
F R	0	10	...	..	0	5	...	...
5	100	107	230	0	0	10	185	40
20	52	65	195	30	A	A	75	110
40	30	45	80	15	A	A	20	95
60	5	17	10	10	A	A	14	100
80	5	18	20	5	0	5	22	60
100	0	10	15	2.5	0	5	13	40
120	0	10	10	0	0	2	15	40

* F R indicates fasting residuum; A, alkaline in the reaction.

ments.[8] The reason, of course, is that the 0.2 per cent strength, ranging a little above the concentration of duodenal toleration (0.15 per cent), is not strong enough to produce marked pylorospasm, but sufficiently irritative to be rejected by the duodenum and so carry back with it duodenal contents.

Certain additional facts which may be worthy of note have presented themselves during the course of these experiments. The first of these concerns the theory of a dissociated regurgitation of bile and pancreatic juice from the duodenum into the stomach—an idea which appears from the literature to be generally accepted, but which has no basis in fact. Any number of papers may be quoted in which this is

accepted on the finding of trypsin in the gastric contents in the absence of visible bile pigment. While anatomic evidence (Baldwin,[14a] Behrend[14b]) shows that an accessory pancreatic duct opens into the duodenum from ¾ to 1 inch higher than the common bile duct, recent physiologic evidence[15] shows that the flow of bile and pancreatic juice into the duodenum is coincident. We have further demonstrated the fallacy of dissociated regurgitation by showing in our experiments that bile pigment in low concentration in gastric juice is not readily recognizable to the naked eye. Bromsulphalein and bile were added separately to portions of gastric juice previously shown to be free of either of these substances. The one containing the dye was made alkaline. Both were then diluted separately until in the one the dye was just recognizable and in the other the bile tinge was just perceptible. The

TABLE 4.—*Relatively Rapid Loss of Acidity in the Gastric Contents in the First Five Minutes as Compared to the Total Lost in an Hour, After the Ingestion of Acid Meals* *

Case	Per Cent Acid Test Meal	Per Cent Acid Lost in		Acid in Ewald Meal
		5 Min.	60 Min.	
1	0.547	20	65	0
	0.200	18	100	
2	0.547	12	76	+
	0.244	7	45	
3	0.490	40	94	—
	0.200	18	82	
4	0.510	27	93	N
	0.220	5	83	

* N indicates normal acid response; +, hyper response; —, hypo response, and 0, achorhydria.

dye concentration was then determined by the usual procedure, while the concentration of bile was determined by the van den Bergh technic. Repeated estimations and calculations of the relative concentrations of the two pigments showed that, on the average, there was required at least five times the concentration of bile as compared to alkalinized bromsulphalein to permit detection by the naked eye with certainty. It seems highly probable, therefore, that previous investigators, believing they had found regurgitated pancreatic juice in the absence of regurgitated bile, were simply erring by using a chemical test for one substance and a visual test for the other, at the same time ascribing

14. (*a*) Baldwin, W. M.: Pancreatic Ducts in Man, Together with a Study of the Microscopical Structure of the Minor Duodenal Papilla, Anat. Rec. **5**:197, 1911; (*b*) Behrend, M.: Surgical Diseases of the Gall Bladder, Liver and Pancreas and Their Treatment, Philadelphia, F. A. Davis Company, 1927, p. 50.

15. McClure, C. W., and Wetmore, A. S.: Studies in Pancreatic Function: Enzyme Concentration of Duodenal Contents After the Ingestion of Pure Foodstuffs and Food Mixtures by Normal Men, Boston M. & S. J. **187**:882, 1922.

equal degrees of sensitivity to both. Dodds and Robertson,[16] recently studying lactic acid in the gastric contents, experienced difficulties which they were able to show were due to occult bile regurgitation.

Another factor which assumed prominence in our work was the rate at which the gastric acidity was lowered. Migai [17] found that a 0.5 per cent solution in the stomach lost 75 per cent of its acidity after an hour, while a 0.3 per cent solution lost 44 per cent, and a 0.1 per cent solution lost about 8 per cent in the same time. Boldyreff [6] cited these experiments as part of the evidence on which he has built up his theory. Migai, however, failed to observe that the percentage of neutralization which occurs in the first five minutes after the introduction of the acid is far out of proportion to the neutralization which takes place in the remainder of the hour, this first five minute neutralization being inexplicable on the basis of Boldyreff's theory. Table 4 illustrates the disproportion in the amount of acid loss during the first five minutes

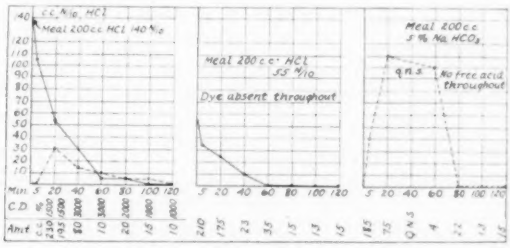


Chart 1.—Graphic picture of the free acidity curve and percentage of dye during the course of the various test meals given to the same patient on successive days under experimental conditions described in the text. In this and in chart 2, *C.D.* indicates the previous determination of the concentration of dye in the duodenum at corresponding times; *Amt.*, the gastric volume at corresponding times; the solid line, free hydrochloric acid, and the broken line, the percentage of dye in the gastric contents.

as compared to the total loss during the first hour after ingestion of the acid meal.

Charts 1 and 2 will deserve a moment's consideration. Chart 1 represents a graphic picture of the free acidity curve and percentage of dye during the course of test meals of 200 cc. each of 140 tenth-normal hydrochloric acid (0.511 per cent), 55 tenth-normal hydrochloric acid (0.20 per cent) and 5 per cent sodium bicarbonate solution, given to the same patient on successive days under the experimental conditions

16. Dodds, E. C., and Robertson, J. D.: Origin and Occurrence of Lactic Acid in Human Gastric Contents, Quart. J. Med. **23**:175, 1930.

17. Migai: Diss., St. Petersburg, 1909; quoted by Bayliss, W. M.: Principles of General Physiology, ed. 4, London, Longmans, Green & Co., 1924, p. 371.

described. The concentrations of dye in the duodenum (C. D.) at corresponding times are noted as well as the total amounts of gastric contents (Amt.) recovered from the stomach at corresponding periods. It will take little mathematics to see what ridiculously small amounts of regurgitation the dotted lines represent, but it will be of interest to note what a greater proportion of regurgitation occurred with the sodium bicarbonate meal, even though no free acid was present throughout; as a matter of fact, the notes on this case show that the gastric contents recovered were for the most part alkaline in reaction.

Were one to examine the graph alone without considering how much actual regurgitation the broken curve really represents, one could

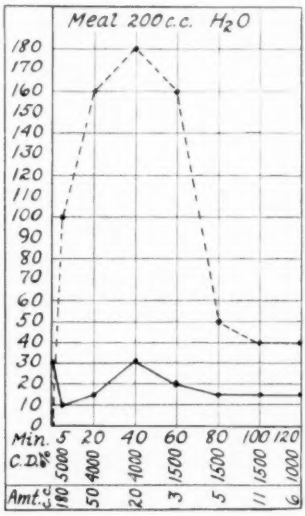


Chart 2.—Graphic representation may be misleading, if the degree of regurgitation is not considered in relation to the concentration of the test substance in the duodenum, to the total gastric contents and to the total test substance present therein at a given time. The apparently high concentration of dye (180 per cent) in the stomach contents at the end of forty minutes fades into insignificance if considered in relation to the foregoing factors.

readily say that a definite increase in regurgitation was noted. Chart 2 makes this point even more obvious. This chart, showing such an apparently large amount of regurgitation, also shows the peak of the regurgitation to correspond with the peak of the acid curve, but when one calculates the actual amount of regurgitation, one sees that less than 2 cc. of duodenal contents would be sufficient to have produced the concentration of dye noted in the stomach.

A brief consideration of the important experimental data that have preceded our studies will now be of interest and their analysis, we think, will help to fortify our contention that duodenal regurgitation in man as well as in dogs is probably a negligible factor in the control of gastric acidity.

Experiments in Which Pancreatic or Duodenal Regurgitation is Prevented.—From his own experiments, Boldyreff [4] stated that the exclusion of the pancreatic juice alone was practically as efficacious in preventing the normal loss of gastric acidity as total exclusion of all juices. This conclusion, based on pancreatic duct ligation in dogs,[18] is not supported by similar experiments of more recent date. Yesko [19] found that the ligation of the pancreatic ducts in dogs caused no striking changes in gastric acidity. Seven dogs similarly treated by Fauley and Ivy [20] showed the average gastric acidity following the operation to be increased in three, a negligible difference in three and actually decreased in one, as compared to that before the operation. Further convincing contrary evidence may be had in the experiments of McCann,[21] who studied the mechanism of gastric acid control in dogs before and after the Mann-Williamson operation. This operation short-circuits all the duodenal contents into the lower portion of the ileum, while the gastric contents pour directly into the upper portion of the jejunum. This procedure makes regurgitation of duodenal contents into the stomach impossible. Postoperative fractional analyses on these animals show no variations from the curves found before the operation so far as they represented the chemistry of digestion or the control of the acidity of the juice. The same adequate control of the gastric acidity occurred after operation as before. He further showed that with the addition of fat (olive oil) to the test meals, the free acid was not controlled any better in the normal dog than in the dog operated on, even though fat may augment the factor of regurgitation in the normal dog. His results suggest that the control of gastric acidity depends essentially on some intragastric mechanism.

McCann [22] further demonstrated how little effect duodenal contents had on gastric acidity by reversing the foregoing experiments; that is, he drained the whole volume of duodenal contents into the fundus of the stomach. Animals so operated on showed the usual characteristic

18. Boldyreff, W.: Einige neuen Seiten der Tätigkeit der Pankreas, Ergebn. d. Physiol. **11**:121, 1911.

19. Yesko, S. A.: The Effects of Ligation of the Pancreatic Ducts on Gastric Secretion, Am. J. Physiol. **86**:483, 1928.

20. Fauley, G. B., and Ivy, A. C.: Effect of Exclusion of Pancreatic Juice on Gastric Digestion, Am. J. Physiol. **89**:428, 1929.

21. McCann, J. C.: Studies on the Control of Acidity of the Gastric Juice, Am. J. Physiol. **89**:483, 1929.

22. McCann, J. C.: Experimental Ulcer, Arch. Surg. **19**:600 (Oct.) 1929.

curves of normal digestion with average normal values for free acid at the end of the period. The same preliminary rise in the value of total acid and neutral chlorides was followed by the usual type of curve for free acid. There was the terminal rise in neutral chlorine in both instances, before and after operation. McCann's work leaves little room for doubt that in dogs, at least, the gastric acid control is independent of anything that may be added from the duodenum.

The closest approach to the same type of experiment in human beings was the work of Baird, Campbell and Hern.[6] These experimenters used the method of double intubation, one tube being in the duodenum, the other in the stomach. Continuous suction was exerted on the duodenal tube, the specimens being segregated into fifteen minute portions, while fifteen minute extractions were made through the gastric tube. Each gastric and duodenal specimen was estimated for free and total acidity and total chlorides. Duplicate experiments were carried out on the same patient; test meals of water, sodium bicarbonate and hydrochloric acid were used. These investigators reasoned that if continuous suction were exerted on the duodenal tube, so that everything entering the duodenum was at once sucked out, regurgitation would be prevented. Thus, in the absence of regurgitation and the failure of neutralization they expected the curves for chlorides and for total acid to be the same. However, they actually found that for the stomach the curves for chloride and for acid remained as widely separated as when the test meals were given and no duodenal suction was applied.

Evidence Based on the Presence of Pancreatic Enzymes in the Gastric Contents.—Numerous experiments have been described presenting the presence of one or the other of the pancreatic enzymes in the gastric contents as irrefutable evidence of duodenal regurgitation. While no doubt true, the interpretation of these findings in relation to the control of gastric acidity is far from convincing to us from our analysis of these reports. Such evidence presented has found its chief support in the work of Spencer, Meyer, Rehfuss and Hawk.[23] They argued that if duodenal regurgitation does occur, one should be able to recognize some of the constituents of the duodenal secretions in the material removed from the stomach. They selected trypsin as the best available test substance, using a method modified by Spencer for its determination. While they do show variations in the amount of trypsin present in the stomach at different times during the digestive phase, their charts fail to demonstrate the extent of regurgitation. At no place do they tell us what the normal pancreatic or duodenal contents show by Spencer's method for trypsin determination. It therefore does

23. Spencer, W. H.; Meyer, G. P.; Rehfuss, M. E., and Hawk, P. B.: Direct Evidence of Duodenal Regurgitation and Its Influences upon the Chemistry and Function of the Normal Human Stomach, Am. J. Physiol. **39**:459, 1915.

not appear likely that the fluctuation in their gastric determinations can be interpreted as a measure of duodenal regurgitation. Their experiments indicate that duodenal regurgitation does take place, but do not necessarily prove that it is an important mechanism in the control of gastric acidity. The trypsin determinations of Medes and Wright [24] are subject to the same criticism. Ehrenreich,[25] from similar studies, was unable to reach any conclusion, because he found trypsin to be present in only thirty-seven of sixty-one instances in the gastric contents. He concluded that the question of duodenal regurgitation is not so simple as Boldyreff suggests. MacLean and Griffiths [26] were unable to show any relationship between the variations in tryptic activity of the gastric contents and the curves of gastric acidity. Ehrmann and Lederer [27] found that trypsin, while usually present in an Ewald meal aspirated after forty-five minutes, was usually low and in no case increased if acid was given with the meal. Their findings are not surprising in the light of experiments showing the effect of irritants on the pyloric mechanism to be detailed in a subsequent publication.

Robitschek [28] reported as confirmatory evidence for the Boldyreff theory the case of a patient who fifteen years previously had swallowed lye, resulting in a complete stricture of the esophagus. A gastrostomy, with the esophagus sutured into the skin, assured no connection between the mouth and the stomach. As evidence of duodenal regurgitation into the stomach, the presence of a diastatic ferment was offered. This was determined by the Wohlgemuth method,[29] which yielded a reading of 3 for the fasting stomach contents and of 42 after a test meal. However, on the same basis, Wohlgemuth reported diastatic figures for pancreatic juice as ranging from 12,000 to 40,000. One can readily see how little actual regurgitation of pancreatic juice the figures of Robitschek represent. Gross [30] reports a similar case, but fails to give any figures for his diastatic values.

---

24. Medes, I. G., and Wright, C. W.: Studies on Duodenal Regurgitation, J. Clin. Investigation **6**:403, 1928.

25. Ehrenreich, M.: Ueber die kontinuierliche Untersuchung des Verdauungsablauf mittels der Magenverweilsonde, Ztschr. f. klin. Med. **75**:231, 1912.

26. MacLean, H., and Griffiths, W. J.: The Factors Influencing the Concentration of Hydrochloric Acid During Gastric Digestion, J. Physiol. **65**:63, 1928.

27. Ehrmann, R., and Lederer, R.: Ueber die Wirkung der Salzsaure auf die Fermentsekretion des Magens und der Bauchspeicheldrüse, Klin. Wchnschr. **45**: 1450, 1909.

28. Robitschek, W.: Ueber physiologische Regurgitation von Pankreassaft in den Magen, Wien. klin. Wchnschr. **35**:604, 1922.

29. Wohlgemuth, J.: Untersuchungen über die Diastasen, Biochem. Ztschr. **9**:10, 1908.

30. Gross, O.: Ueber den physiologischen Rückfluss von Pankreassaft in den Magen, Arch. f. klin. Med. **132**:121, 1920.

A Mathematical Consideration of Duodenal Regurgitation; Reaction in the Duodenum.—It is difficult to understand how regurgitation from such an area as the duodenum which has recently been shown to be more often acid than alkaline in reaction,[31] can materially influence the acidity of the stomach. Martin,[32] from his studies of duodenal reaction, concludes that the normal duodenal contents are nearly neutral, but under any digestive procedure, are likely to become acid. Our own experiments, to be detailed later, in which the duodenal contents containing large quantities of bromsulphalein were collected, showed these contents to be, in most cases, neutral or acid, since the dye was not discernible until the material was alkalinized. Such was also the case in three instances of true achylia gastrica (histamine). The duodenal contents collected from these patients, containing large quantities of bromsulphalein which had been previously injected intravenously, failed to give any evidence of its presence until alkali was added. This is particularly significant when we consider that no acid could have been added from the stomach in these patients. This fact becomes still more striking if we recall that bromsulphalein ceases to be colorless at a p_H of 7.2.

Setting aside, for the moment, this evidence, let us see what amounts of regurgitation would be necessary to satisfy the experiments presented in support of the Boldyreff theory. The majority of these experiments involved the introduction of 0.5 per cent hydrochloric acid into the stomach in amounts ranging between 100 and 200 cc. We used 200 cc. quantities in our experiments. Assuming that Boldyreff's contention that 0.15 per cent hydrochloric acid is the upper limit of concentration at which hydrochloric acid is acceptable to the duodenum, granting that the pancreatic juice is the most alkaline secretion reaching the duodenum, admitting the alkalinity of pancreatic juice to be 0.65 per cent sodium carbonate as quoted by Boldyreff [4] (though this is stronger than recently found by Ivy [11]), it would still take approximately 120 cc. of *undiluted* pancreatic juice to reduce 200 cc. of 0.5 per cent hydrochloric acid to 0.15 per cent, allowance already being made for the dilution reduction in the stomach. This amount would have to be delivered to the stomach in a comparatively short time, about one hour, to produce the desired result. Ivy [11] has shown that the greatest degree of secretory stimulation possible in a dog's pancreas is about 70 cc. per hour, an amount far short of that required in the animal experiments, and certainly in our human experiments if considered on

31. Mann, F. C., and Bollman, J. L.: The Reaction of the Content of the Gastro-Intestinal Tract, J. A. M. A. **95**:1722 (Dec. 6) 1930.

32. Martin, L.: The Hydrogen Ion Concentration of Successive Portions of Duodenal Contents Following Stimulation with Magnesium Sulphate, Arch. Int. Med. **39**:275 (Feb.) 1927.

a proportionate basis. The diluting effect of the other intestinal juices is also disregarded in the foregoing calculations. The Boldyreff theory would also assume considerable addition of fluid to the gastric contents before emptying occurred. Such is not borne out in our table 1 showing the rapid drop in acidity in a five minute period, with comparatively small changes in volume of the gastric contents.

Experimental Evidence Based on the Curves for Gastric Chlorides.—Bolton and Goodhart [33] estimated the total chlorides as a measure of gastric secretion and the inorganic chlorides as a measure of the amount of duodenal regurgitation. They showed that the curve of inorganic chlorides gradually rises from the beginning of digestion, with that of the active hydrochloric acid, to more or less the same level. This rise they attribute to the neutralization of some of the hydrochloric acid secreted by what alkali happens to be present in the food, swallowed in the saliva or contained in the gastric mucus or pyloric juice, as well as to the decomposition of organic salts in the food by the hydrochloric acid. At the point at which the curve of active hydrochloric acid falls, the curve of inorganic chlorides continues to rise sharply, following that of the total chlorides at a somewhat lower level. This second rise they attribute to neutralization of the hydrochloric acid by the regurgitated duodenal juice.

While one does not deny the aforementioned series of changes in their relation to each other, subsequent experimental evidence indicates that the foregoing explanation of the phenomena is certainly at fault. Thus, MacLean and Griffiths,[26] who studied the curve of gastric chlorides as well as trypsin in the gastric contents, were unable to demonstrate a rise in trypsin content parallel with the marked rise in neutral chlorides and subsequent fall of acidity. Of still greater significance is the demonstration of the foregoing relative changes of neutral chlorides and free acid in the secretions of a Pavlov pouch in dogs into which no duodenal regurgitation could occur. This was shown by MacLean, Griffiths and Williams.[34] Finally, incontrovertible evidence in this respect is again available in the work of McCann,[22] who found the same terminal rise in neutral chlorine and drop in hydrochloric acid both before and after his short-circuiting operations.

CONCLUSIONS

Granting that regurgitation from the duodenum into the stomach frequently occurs, we differ strongly from Boldyreff in the interpreta-

33. Bolton, C., and Goodhart, G. W.: Duodenal Regurgitation into the Stomach During Gastric Digestion, Lancet **1**:420 (March 4) 1922.

34. MacLean, H.; Griffiths, W. J., and Williams, B. W.: Variations in the Acidity and Total Chloride Contained in the Secretion from an Isolated Pavlov Pouch in the Dog, J. Physiol. **65**:77, 1928.

tion and significance of this regurgitation basing our contention on the following evidence:

1. The introduction of weaker acids (0.2 per cent hydrochloric acid) into the stomach frequently produces greater amounts of regurgitation than do stronger acids (0.5 per cent).

2. Ingestion of alkalis (1.0 and 5.0 per cent sodium bicarbonate) often produces more marked regurgitation than either of the aforementioned acids, even though the gastric contents remain alkaline or anacid throughout the test period.

3. The greatest amount of regurgitation noted during all of our experiments occurred in a case of true achylia gastrica during the course of a plain water meal.

4. The reaction of the duodenal contents is such that even if considerable regurgitation did take place, it could not be efficacious in lowering gastric acidity.

5. The duodenal contents are not sufficiently alkaline to change the color of bromsulphalein, a dye which changes color at a p_H of 7.2, this even in cases of true achylia gastrica.

6. The amount of pancreatic juice necessary to produce the reductions in gastric acidity noted is far beyond the ability of any pancreas to secrete during the time allotted.

7. Dissociated regurgitation of pancreatic juice and of bile is based on a fallacy in experimentation.

8. With the introduction of acids into the stomach, some very rapid mechanism is brought into play. This mechanism being neither one of neutralization nor of dilution, we believe is very likely one of absorption.

Therefore, as a result, we have been unable to ascribe any significance to duodenal regurgitation in relation to gastric acid control. We are in agreement with McCann, whose results suggest that this control depends essentially on some intragastric mechanism. From the experiments cited in this paper and from roentgenographic evidence to be detailed elsewhere, we are inclined to look on duodenal regurgitation as part of the pyloric mechanism.

265 South Nineteenth Street.

INTRINSIC GASTRODUODENAL LESIONS AS CAUSATIVE FACTORS OF HEMATEMESIS

ANDREW B. RIVERS, M.D.
AND
DWIGHT L. WILBUR, M.D.*
ROCHESTER, MINN.

Previous study [1] has revealed that intrinsic gastroduodenal lesions are responsible for approximately 90 per cent of all cases of hematemesis. Since hematemesis is usually produced by such lesions, it is justifiable to consider their diagnostic significance in detail, for an accurate diagnosis is highly desirable and is the key to eventual successful treatment. The outstanding factor of value in the differential diagnosis of such intrinsic lesions is the correlated consideration of (1) an accurately recorded and perused history and (2) laboratory studies, chiefly roentgenologic observations. Among intrinsic gastroduodenal lesions producing hematemesis, the most common are peptic ulcer and gastric carcinoma, while nonspecific inflammatory lesions, mucosal erosions, benign tumors, gastric syphilis and tuberculosis make up a small proportion of hemorrhagic lesions. This enumeration of some of the intrinsic gastroduodenal diseases producing hematemesis reveals that they are of almost equal medical and surgical significance, and, consequently, cooperative treatment is essential. It has been pointed out that it is usually not essential that an accurate diagnosis be made before treatment is begun, since relatively simple medical measures will usually be sufficient to control the bleeding producing hematemesis. The precipitation of hasty surgical treatment is frequently ineffective in controlling this bleeding and may jeopardize rather than increase the patient's chances.

Balfour,[2] Miller,[3] Bastedo [4] and others have pointed out the great frequency of intrinsic gastroduodenal lesions as the causes of hema-

From the Division of Medicine of the Mayo Clinic and the Mayo Foundation.
* Fellow in Medicine, the Mayo Foundation.

1. Rivers, A. B., and Wilbur, D. L.: The Diagnostic Significance of Hematemesis, J. A. M. A. **98**:1629 (May 7) 1932.

2. Balfour, D. C.: Hematemesis, Tr. Coll. Phys. **44**:236, 1922.

3. Miller, T. G.: Gastric Hemorrhage from the Internist's Point of View, Pennsylvania M. J. **32**:237 (Jan.) 1929.

4. Bastedo, W. A.: Medical Measures Used in the Treatment of Hematemesis, Tr. Am. Gastro-Enterol. A. **30**:107, 1927; M. J. & Rec. **126**:333 (Sept. 21) 1927.

temesis. It seems almost universally accepted that peptic ulcer is the chief cause of vomiting of blood, and yet, as recently as 1930, Hughes [5] attempted to discredit this belief and stated that, in twenty-five years of experience, he had observed only two cases of true hemorrhage as a result of peptic ulceration. The earlier studies of White [6] concerning gastrostaxis seem worthy of revaluation in the light of recent observations and the present benefits of more accurate studies now possible. Consideration of the incidence, mechanism and diagnosis of the various causes of hematemesis will be included.

MATERIAL FOR STUDY

The present study includes the data obtained from a review of the histories of all patients who came to the Mayo Clinic complaining of vomiting of blood during the years 1927 and 1928. Those giving a history solely of melena were not included. Since the tabulation of the data in cases studied in 1928 represents an almost exact reduplication of those tabulated for 1927, there is assurance that the estimations presented are reasonably accurate. Our material includes 668 cases, in 236 of which diagnosis was made after careful clinical investigation, without surgical exploration, and in the remaining 432 cases, we have the additional information obtained through careful surgical exploration. In 602 (91 per cent) of these 668 cases, hematemesis was due to intrinsic gastroduodenal lesions. Except that relatively few cases in which acute infectious diseases are accompanied by hematemesis reach us for diagnosis, and that perhaps a larger proportion of surgically treated peptic lesions are represented, the data obtained should not be at great variance with those observed in the general practice of medicine.

RESULTS

A summary of the results of the study undertaken in 602 cases of intrinsic gastroduodenal lesions as causative factors of hematemesis is presented in table 1; their relative significance is tabulated graphically in table 2. Cases in which exploration was done and those in which it was not done are reviewed in table 3. There is no great difference in the types of cases represented among the groups in which exploration was done and those in which it was not done, although, as would be expected, in the latter group in some instances the diagnosis could not

5. Hughes, Basil: Hematemesis as a Symptom of Gastric or Duodenal Ulcer, Lancet **2**:1346 (Dec. 20) 1930.

6. White, W. Hale: An Address on Gastrostaxis, or Oozing of Blood from the Mucous Membrane of the Stomach, Lancet **2**:1189 (Nov. 3) 1906; Some Forms of Haemorrhage Which Are Difficult of Explanation, ibid. **1**:416 (Feb. 17) 1912.

be as positively established as in those in which surgical intervention was performed. This is particularly true of the group classified as having a history of ulcer, but roentgenologically negative. A positive diagnosis of ulcer was not made in these cases in the absence of roentgenologic confirmation. Undoubtedly, exploration would have placed

TABLE 1.—*Incidence of Lesions*

Lesion	Number	Per Cent
Duodenal ulcer	356	59.20
Gastric ulcer	43	7.10
History of gastric ulcer, but roentgenologically negative	22	3.60
Secondary ulcer	92	15.30
Carcinoma of stomach	84	13.80
Adenoma of duodenum	1	0.16
Myoma of stomach	1	0.16
Gastric syphilis	1	0.16
Cholecystoduodenal fistula	2	0.32

TABLE 2.—*Relative Frequency of Intrinsic Gastroduodenal Lesions as Compared to Other Causes of Hematemesis*

Lesion	Number	Per Cent
Ulcer	513	76.80
Carcinoma	84	12.60
Other benign lesions	5	0.83
Other causes	67	9.65

TABLE 3.—*Incidence of Lesions in Explored and Unexplored Cases*

Explored	Number	Per Cent
Duodenal ulcer	245	56.6
Gastric ulcer	33	7.7
Secondary or reactivated ulcer	53	12.7
Carcinoma of stomach	56	13.0
Adenoma of stomach	1	0.2
Myoma of stomach	1	0.2
Gastric syphilis (with hepatic involvement)	1	0.2
Cholecystoduodenal fistula	2	0.4
Unexplored		
Duodenal ulcer	111	47.0
Gastric ulcer	10	4.2
History of ulcer, but roentgenologically negative	22	9.3
Secondary or reactivated ulcer	39	16.5
Carcinoma of stomach	28	11.9

some of the cases in the ulcer group. The pathologic processes that may be responsible for bleeding in some cases of apparent peptic ulcer are such that it is not surprising that the lesion at times is not demonstrable roentgenologically, although at the time the present study was made (from 1927 to 1928) the diagnosis of peptic ulcer by this method had reached a high degree of accuracy. The presence of localized or diffuse areas of inflammatory reaction, of nonspecific character, in the stomach or duodenum or surrounding a gastro-enteric stoma, may lead not only to symptoms suggestive of peptic ulceration but also to hemor-

rhage, as demonstrated by one of us (Dr. Rivers [7]), and yet such areas may not produce sufficient abnormality in the contours or functions of these organs to be recognizable roentgenologically. In addition, the failure of the roentgenologist or the surgeon to find evidence of ulceration in the stomach or duodenum at the time of examination may be due to the fact that such examination is carried out several weeks after the occurrence of the bleeding. During this interval it is conceivable that many small lesions will heal completely.

PEPTIC ULCER

It is apparent that peptic ulcer is the greatest offender in the causation of hematemesis, since more than 75 per cent of the total number of cases in which this symptom occurred presented evidence of duodenal, benign gastric or anastomotic ulceration. Similarly, peptic ulcers were responsible factors in more than 85 per cent of the cases of hematemesis due to intrinsic gastroduodenal lesions.

It is generally considered that approximately 20 per cent of all duodenal ulcers bleed. The clinical varieties and exciting causes of bleeding are many; the bleeding may be occult or gross, large or small in amount, and may occur as melena or hematemesis, or both. Primary hemorrhage is rarely fatal. It has been stated by others that hematemesis in the presence of a peptic ulcer is usually an indication of the presence of gastric ulcer, and that duodenal ulcers rarely provoke vomiting of blood. On the contrary, if they bleed, melena is the expected result. Our experience does not bear out this statement. Matthews [8] expressed the belief that the bleeding of a duodenal ulcer is more serious than that of gastric ulcer, because the bleeding is more severe and more continuous.

Ulcers on the anterior wall of the duodenum bleed less frequently than ulcers of the posterior wall because of the less abundant supply of blood to the anterior wall, which is relatively anemic. The posterior wall of the duodenum is more vascular, and ulcers in this area more readily penetrate into the pancreas, which is quite vascular. In the present series, duodenal ulcers accounted for 356 of the 602 cases (59 per cent) of hematemesis due to intrinsic gastroduodenal lesions. In an additional 22 cases, the history was positive for ulcer, but the roentgenologic examination was not confirmative.

The mechanism in the production of the bleeding may depend on a distant cause in addition to the local lesion of the duodenum. Although

7. Rivers, A. B.: A Clinical Study of Duodenitis, Gastritis and Gastrojejunitis, Ann. Int. Med. **4**:1265 (April) 1931.

8. Matthews, Edwin: The Clinical Significance of Hematemesis in Gastro-Intestinal Diseases, New York, Oxford University Press, 1928, p. 120.

the essential underlying cause of the bleeding is rupture of a blood vessel, the type of vessel ruptured and the agent provoking the rupture may vary considerably. Three types of vessels may produce such bleeding: 1. A moderate-sized or a large vessel may be penetrated as a direct result of the process of peptic ulceration; such vessels are frequently sclerotic and lie at the base of the ulcer, which generally is in the posterior wall of the duodenum, or which has perforated through into the pancreas. Rarely, a large vessel, such as the pancreaticoduodenal and the splenic, or a large gastric vessel may be ruptured, and under such circumstances the bleeding may rapidly become of serious significance. Bleeding from this type of vessel is usually extensive and frequently produces hematemesis of bright red blood in large amounts, followed by melena. 2. This type is comprised of the group of small, actively and passively congested vessels which surround an acutely or subacutely inflamed area, and which are easily ruptured by trauma, by excessive venous stasis or by changes in the blood itself. Bleeding under such circumstances is usually slow and oozing, but the loss may be considerable. Although hematemesis is less common as a result of this type of lesion, it nevertheless occurs; melena is the more common result. 3. Vessels in the buds of vascular granulation tissue in the base of the ulcer, formed during the process of healing, may bleed profusely when the buds break off. Associated disease in other organs which produces venous stasis of the stomach or duodenum may produce conditions favorable for bleeding from a peptic ulcer, which, in the absence of such stasis, would not necessarily bleed.

Among the more common exciting factors productive of bleeding from gastroduodenal lesions may be mentioned excessive physical exertion, acute infection, alcohol, fatigue or emotional strain and hypersensitive types of reaction. It is well known that physical strain, particularly heavy lifting, will provoke bleeding from an ulcer, which is presumably due to the associated elevation of both arterial and venous pressure, with rupture of a vessel within the ulcer. Acute infections or exacerbations of chronic infections, particularly of the mouth and upper part of the respiratory tract, may be productive of gastrointestinal bleeding, probably as a result of associated lighting up of the inflammatory process surrounding the ulcer. Such changes leading to the bleeding have been demonstrated clinically by bouts of bleeding from ulcer following extraction of teeth or tonsils, and experimentally by the work of Rosenow.[9] Alcohol, by its direct irritation of the ulcer or by stimulation of excessive gastric secretion, provokes bleeding. Fatigue and emotional strain frequently provoke sufficient flare-up

9. Rosenow, E. C.: The Causation of Gastric and Duodenal Ulcer by Streptococci, J. Infect. Dis. **16**:333 (Sept.) 1919.

of the ulcer to result either in further penetration, which may include a blood vessel, or else exacerbation of the inflammatory reaction surrounding the ulcer. Burns and hypersensitive types of reaction are among the rare causes of bleeding from an ulcer. It is well known that extensive burns may lead to acute ulcer of the duodenum, the so-called Curling's ulcer, which may bleed, or bleeding may occur without any actual ulceration and may be so extensive as to be a factor in producing a fatal result. This recently occurred in a case under our observation. It is to be pointed out that peptic ulcer may be present coincidentally with many other diseases, for example, cirrhosis of the liver, coronary thrombosis, diabetes, tabes with gastric crisis, and so forth, which have been reported as leading to gastric hemorrhage, and it may be that the associated ulcer is the actual cause of such bleeding.

Occasionally, a bleeding ulcer on the posterior wall of the duodenum will not produce sufficient change to be demonstrable by inspection of the duodenum at operation. Incision of the anterior wall of the duodenum, with exposure of the mucosal surface of the posterior wall, will occasionally reveal an ulcer which is the cause of previously considered, unexplained bleeding. This point has been emphasized by Balfour.

It is considered that gastric ulcers bleed in approximately 25 per cent of all cases. In the present series, gastric ulcer accounted for 43 (7.1 per cent) of the 602 cases. This, compared to 59 per cent for duodenal ulcers, indicates that bleeding duodenal ulcers occurred eight times as frequently as bleeding gastric ulcers in the present series. This approximates the percentage incidence of the 2 lesions.

The mechanisms of production of bleeding in gastric ulcer are similar to those already considered for duodenal ulcer. Also, a gastric ulcer undergoing malignant change almost invariably causes occult blood in the stools and gastric content, whereas benign ulcer, as a rule, leads to occult bleeding in periodic cycles, coincident, apparently, with the activity of the ulcer.

This series includes 92 cases in which the hemorrhage leading to hematemesis was found to be due to secondary peptic ulceration. It is an interesting fact, as has been previously pointed out by one of us (Dr. Rivers), that in cases of peptic ulcer in which there has once been hemorrhage there seems to be a special tendency to occurrence of bleeding, if recurring ulcers or reactivation of such ulcers occur following medical or surgical treatment. If, for instance, an ulcer exhibiting hemorrhagic tendencies is present in the duodenum, for the cure of which short-tracking operations were performed, there seems to be a special liability on the part of the symptoms, if they

recur, to include further bleeding. Surgical treatment, therefore, in cases in which there is hemorrhage should include, whenever possible, the excision of such lesions.

Our present series includes certain cases in which the duodenal ulcer was excised and in which a second ulcer exhibiting hemorrhagic tendencies appeared in the duodenum. A few cases have also come under our observation in which gastric ulcer was found to have been the cause of bleeding, and in which, after excision of the ulcer and gastroenterostomy, further hemorrhage took place. This may be found to be due to a second ulceration in the stomach, which was too small to be readily recognized at the time of the first operation. It is therefore essential to explore the entire stomach, even though one ulcer is found which might be held responsible for symptoms in a given case.

Gastrojejunal types of ulceration are likely to be complicated lesions, the two types of complications most frequently encountered being hemorrhage and deep penetration or even subacute or acute perforation. Hemorrhage occurs in 34 per cent of the cases of gastrojejunal ulcer. Occasionally, hemorrhages arise from shallow, mucosal erosions surrounding the gastro-enteric stoma. At other times, extensive lesions about or below the stoma are found, from which extensive bleeding occurs. Gastrojejunal ulcers are a little more likely to cause bleeding by bowel than to produce hematemesis, and the bleeding is likely to be slow, so that long-continued oozing may occur before the complication of the bleeding is suspected.

The diagnosis of ulcer is generally simple since, in the majority of cases, a good history of peptic ulcer is obtained. Careful questioning may elicit such a history in an additional number of cases in which it at first appeared that there was no history of such a complaint. It is unusual for the bleeding to occur without any preceding gastro-intestinal disorder. Hinton[10] pointed out that the cases of acute hemorrhage that proved fatal in spite of conservative treatment occurred in cases in which the history was negative or short. This observation deserves further consideration. The fatal cases in the present series were so few that denial or affirmation cannot be made. Roentgenologic study, when the bleeding has ceased, will usually localize the lesion in the stomach or duodenum. Such localization by history alone is, as a rule, unsatisfactory.

The differential diagnosis of the various recurring or reactivated duodenal ulcers offers a study which, in itself, is sufficiently extensive to warrant separate consideration. We shall mention here merely a few of the fundamental facts regarding diagnosis. In recurring ulcera-

10. Hinton, J. W.: Bleeding Gastric and Duodenal Ulcers: Report of Fifty-Two Cases, Ann. Surg. **93**:949 (April) 1931.

tions, if the secondary lesion is in an area approximating the first lesion, the symptoms including the site of pain are usually similar to those experienced with the original lesion. If recurrence takes place in the duodenum, the symptoms generally are almost exactly like those experienced previous to the first operation. These principles apply also in cases of recurring gastric ulcer in which secondary ulcer develops in an area approximating the original area. The syndrome of a gastrojejunal ulcer usually includes pain farther to the left and considerably lower than that experienced with the original ulcer in the stomach or duodenum. Roentgenologic examination still offers great help in the localization, although, obviously, in recurring ulcers of the duodenum, its efficiency is not so great as in the case of the original lesion. In such instances the history, however, is usually sufficiently definite so that its careful study will permit of a diagnosis without great difficulty.

HISTORY OF ULCER ROENTGENOLOGICALLY NEGATIVE, NOT EXPLORED

This group, which includes 22 cases, seems of sufficient importance to be considered separately. A positive diagnosis was not made in these cases, although there seems little doubt that a diagnosis of peptic ulcer usually would have been justifiable. Although it is not an infallible rule, it is generally true that history which includes data on hemorrhage and indigestion, with characteristics of ulcer, is diagnostic of an intrinsic, ulcerating or localized inflammatory gastroduodenal lesion, regardless of the absence of confirmation by the roentgen rays. Such lesions may be malignant, but more often they are benign.

The cases in which exploration was done include many in which the roentgenologic examination for a lesion was not conclusive or failed to show any deformity, and yet operation was advised because the symptoms seemed sufficient to make exploration advisable. In certain such cases, operation was not advised because the history included evidence suggesting only trivial amounts of bleeding or a mild type of indigestion. In other cases, there was a history of rather brisk hemorrhage, which had occurred months or even years prior to consultation at the Mayo Clinic, and in these merely a mild type of indigestion persisted. Operation was not advised in others because of the presence of some serious intercurrent disease, so that operation would have been undertaken at considerable risk to the patient.

There is still another group of cases in which operation was advised but refused by the patients. On several occasions, it has been pointed out by one of us (Dr. Rivers) that serious indigestion, which may be associated with hemorrhages, can be produced by shallow mucosal lesions with or without ulceration in the stomach, duodenum or about a gastroenteric stoma. One need only inspect such lesions in the operating room

to be convinced that roentgenologic evidence might be entirely lacking. In such cases there may be no lesion capable of producing a niche other than an erythematous, inflamed mucosal or submucosal area, in which there may or may not be a few small, punched-out areas, and it is conceivable that these lesions would not produce direct roentgenologic evidence of their presence.

Kirklin,[11] in describing the appearance of shallow duodenal lesions, stated that occasionally one could recognize such lesions by the extreme irritability of the duodenal bulb, even though there was no direct evidence of a fleck or a niche. The liability of such lesions to bleed is indicated by the fact that gastro-enteric hemorrhage occurred in 15 per cent of cases of duodenitis reported by one of us (Dr. Rivers) in 1931. There seems little doubt that these shallow inflammatory lesions go a long way toward explaining most of the so-called indeterminate cases of hemorrhage. It is probable that in many of the cases of so-called gastrostaxis, as described by Hale White and others, the hematemesis is due to such nonulcerating inflammatory lesions. If operation were undertaken during such hemorrhage or immediately following, there would be definite evidence, in most instances, of the presence of some such lesion if any other cause for the hemorrhage could not be demonstrated.

CARCINOMA OF THE STOMACH

Malignant lesions involving the stomach were found to be the cause of hematemesis in 12.6 per cent of all cases coming under our observation over a two year period. Although, undoubtedly, most carcinomas of the stomach are responsible for some bleeding, it is unusual that gross bleeding occurs. A careful investigation of the stool in most instances will reveal the presence of small amounts of occult blood, which are present rather consistently from day to day. The vomiting of blood in large amounts is, however, an extremely rare complication of carcinoma of the stomach. This symptom was present in only 1 per cent of a series of cases of gastric carcinoma. The amount of blood vomited is usually small and, because of its mixture with gastric content, it resembles coffee-grounds.

In many instances of gastric malignancy, the diagnosis cannot be made from careful evaluation of the history, and if the diagnosis is delayed until vomiting of blood occurs, the majority of these patients will have passed beyond that period when the surgical removal of such lesions is still possible.

The diagnosis of early carcinoma of the stomach is usually made by means of roentgenologic investigation. This method of examination is

11. Kirklin, B. R.: A Roentgenologic Consideration of Duodenitis, Radiology **12**:377 (May) 1929.

indispensable in confirmation of the clinical diagnosis of gastric carcinoma, and in addition localizes the position and extent of the lesion, thus giving information which is valuable in determining whether the lesion is resectable. The development of indigestion late in life is always suggestive of a malignant condition and should lead to roentgen investigation.

Bleeding in cases of gastric carcinoma is usually the result of ulceration of the wall of the stomach or of necrosis and sloughing of papillary growths. Consequently, it is the ulcerating and papillary growths that most frequently bleed. Infiltrating lesions of the linitis plastica type do not produce gross hemorrhage as a rule. Because of the small vessels involved, the bleeding is usually an oozing or seepage in relatively small amounts. As a result, action of gastric juice on the blood occurs, and the typical coffee-grounds material (acid hematin) is produced. If excessive hemorrhage occurs, it is usually the result of direct, malignant ulceration into a medium-sized or large vessel, and is usually a part of the terminal picture of gastric carcinoma when the disease has become extensive.

INFREQUENT INTRINSIC LESIONS RESPONSIBLE FOR HEMATEMESIS

Cholecystoduodenal Fistula.—Under this heading two cases are included in which fistulas between the gallbladder and the duodenum were considered significant factors in the causation of hematemesis. In one case, the fistula was undoubtedly the result of an ulcer of the duodenum perforating through into the gallbladder; in the other the original lesion was in the gallbladder, and subsequently the duodenum was perforated and invaded by a gallstone.

The history in the first case included evidence suggesting peptic ulceration, and the roentgenogram showed a definite deformity, such as that caused by ulcers of the duodenum. The bleeding was extensive. It is extremely unusual that a fistula between the duodenum and gallbladder is formed in this way. There had evidently been an extensive amount of periduodenal inflammation, with fixation of the gallbladder to the duodenum. Subsequently, there had been progressive involvement of the wall of the duodenum by means of ulceration, with burrowing through the serosa and eventually through the wall of the gallbladder.

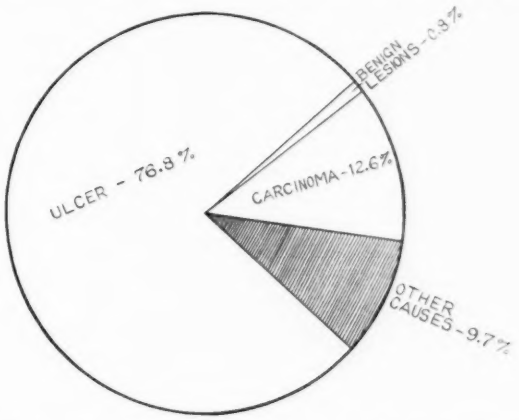
In a series of cases recently studied, one of us (Dr. Rivers) and Mason[12] found that there were only 2 of 60 cases in which the original lesion, resulting eventually in cholecystoduodenal fistula, was found to be in the duodenum. Usually this complication is produced by extension

12. Rivers, A. B., and Mason, J. B.: Cholecystoduodenal Fistula, M. Clin. North America **15**:1571 (May) 1932.

of an inflammatory reaction surrounding an acutely inflamed gallbladder, and the duodenum becomes fixed in the resulting adhesions. There is a minute rupture of the gallbladder, with formation of abscess, and a stone escapes from the gallbladder into the abscess. From this intermediary fistula there is extension subsequently of the inflammatory process and passage of the stone through the wall of the duodenum. Occasionally, the rupture takes place without an intermediary fistula. Bleeding in such cases is due to the ulceration and inflammatory reaction in the duodenum, gallbladder or intermediary fistula.

BENIGN TUMORS OF THE STOMACH AND DUODENUM

CASE 1.—*History.*—A woman, aged 64, complained of an aching type of epigastric pain, which she had noted for two years. This pain was not constant



Percentage of occurrence of gastroduodenal lesions as causative factors of hematemesis.

but would come on at intervals, lasting a week or longer. During these intervals, gaseous indigestion was present. With one of the earliest episodes of indigestion she had vomited large amounts of blood. In the two years subsequent to the onset of this complication, she vomited a number of times, so that the amount of blood lost must have been exceedingly great. She was fed a bland diet and was quite comfortable on this regimen. At frequent intervals, however, there had been mild, dull, aching pain in the epigastrium, which came on at any time following a meal. Soda or food did not relieve this. She weighed 201 pounds (91.1 Kg.).

Examination.—The blood pressure in millimeters of mercury was 165 systolic and 90 diastolic. General examination revealed nothing of importance. The concentration of hemoglobin was 55 per cent; the erythrocytes numbered 2,500,000 and leukocytes 4,100 in each cubic millimeter of blood. Estimation of gastric acids revealed that free hydrochloric acid was 10. The amount expressed was not unusual. Some fresh blood was included in the expressed material. Roentgenograms revealed the presence of a tumor lying high in the stomach.

Course.—At operation, a myoma high on the posterior wall, near the cardiac portion of the stomach was found. The tumor was attached to the stomach by means of a pedicle. There was an ulcerated area on the surface of the myoma. The tumor was excised. Convalescence was without incident. .

CASE 2.—*History.*—A man, aged 37, had had periods of pain in the epigastrium which came on two hours after meals. The period of pain would last for several weeks, and then for several months he would be entirely free from symptoms, which consisted of what he described as a dull, gnawing pain, which was relieved by eating. About eight weeks before consultation at the clinic, the pain became severe and lasted for a day or two. Five weeks before registration, he had had another episode of severe pain and shortly after this had felt nauseated and had vomited large amounts of dark, bloody material. He consulted his physician, who made a diagnosis of peptic ulcer and advised a regimen of milk and alkali. Since the institution of this type of treatment, the patient had been quite comfortable.

Examination.—Examination disclosed slight tenderness in the epigastrium. There were no palpable masses. Estimation of gastric acids showed total acidity 62, and free hydrochloric acid 44; the amount expressed was 350 cc. The concentration of hemoglobin was 73 per cent; erythrocytes numbered 4,650,000, and leukocytes 7,600. There was no evidence of enlargement of the spleen or of disease of the liver. Roentgenologic investigation of the stomach and duodenum failed to show any definite evidence of abnormality. Because of the history, which included gastro-intestinal hemorrhage, operation was advised.

Course.—A chronically diseased appendix and a small tumor of the duodenum were found. The pathologist reported this to be a myoma. It was assumed that the bleeding came from the tumor. The patient was examined two years after operation, and there had been no recurrence of the bleeding.

Benign tumors of the stomach and duodenum are rare, and they do not as a rule cause gross bleeding. The most common benign tumors involving the stomach and duodenum are polyps, adenomas, myomas, fibromas, hemangiomas and papillomas. The symptoms arising from these tumors may be of several types, for example: (1) those caused by interference with normal mechanics of digestion, such as difficulties in proper emptying of the stomach or obstruction by tumors becoming impacted in the pylorus or the lumen of the bowel, and (2) those arising subsequent to severe anemia, which may be associated with these tumors. Balfour and Henderson,[18] in reviewing the symptoms in a series of benign gastric and duodenal tumors, felt that the most common and most significant sign of benign tumor of the stomach is anemia. They pointed out that, although benign tumors seldom bleed, if they do, the loss of blood may be extremely exhausting.

The pathologic changes in benign gastric and duodenal neoplasms leading to bleeding are variable and depend on the nature of the tumor. Polyps continually oozing small amounts of blood, because of superficial necrosis and sloughing, may lead to severe anemia which may be

13. Balfour, D. C., and Henderson, E. F.: Benign Tumors of the Stomach, Ann. Surg. **85**:354 (March) 1927; Benign Tumors of the Duodenum, ibid. **89**:30 (Jan.) 1929.

recognized as secondary anemia or occasionally as anemia with a picture simulating primary (pernicious) anemia. It may be difficult to believe that severe anemia can be produced by such a small lesion, especially in the absence of manifest, gross bleeding. Rieniets [14] stated that bleeding in cases of gastric leiomyoma is characteristically periodic and profuse, and that it depends on deep ulcerations of the tumor. The larger the tumor, the more readily does profuse hemorrhage occur. The pathogenesis of bleeding in cases of hemangioma is obvious.

Pyloric obstruction was found to be present in 10 per cent of the cases reported by Balfour and Henderson. In obstructed cases the tumors were found to be attached to the posterior wall of the stomach, and, because of either a pedicle or redundant mucosa, could occlude the pyloric canal. They pointed out that in certain instances, the indigestion associated with these tumors had definite characteristics of peptic ulceration. Preoperative diagnosis is made almost exclusively by fluoroscopic examination.

GASTRIC SYPHILIS

CASE 3.—*History.*—A man, aged 27, stated that ten years prior to his visit to the clinic, he had vomited blood on one occasion. Eight years later, severe pain in the left upper part of the abdomen had developed which lasted for half an hour. It was necessary to relieve this pain by the use of morphine. He had been hospitalized for three weeks' observation, but a definite diagnosis was not made. At that time, he noticed black stools for a few days. Following this episode, he was well for a period of one year, at the end of which time distress again developed in the epigastrium. This time the pain was not so severe, and he noticed much flatulence and a sensation of fulness in the epigastrium after meals. At intervals, he had pain in the left upper part of the abdomen, and on a few occasions there was some vomiting. His distress usually came on during or immediately following meals, and occasionally he vomited shortly after a meal.

Examination.—Examination disclosed a palpable mass in the upper left part of the abdomen. The concentration of hemoglobin was 50 per cent; the erythrocytes numbered 3,950,000, and leukocytes 8,600. Estimation of gastric acids showed total acidity ranging from 36 to 96 and free hydrochloric acid from 16 to 76. The serologic test for syphilis was positive. Tests of hepatic function did not give evidence of retention of dye. The serum bilirubin was within normal limits. Roentgenologic examination disclosed an hour-glass stomach, and a diagnosis was made of gastric ulcer.

Course.—Exploration revealed the presence of a gastric lesion which had all the characteristics of syphilis. There were several good-sized nodules in the liver, one of which was excised for diagnosis and proved to be inflammatory. There was fairly definite obstruction between the loculi of the hour-glass contraction, and gastrogastrorrhaphy was performed. Subsequent to the operation, the patient was given intensive treatment for syphilis. There has been no evidence of recurrence of the hemorrhage.

---

14. Rieniets, J. H.: The Frequency and Pathologic Aspects of Gastric Leiomyoma, Proc. Staff Meet., Mayo Clin. **5**:364 (Dec. 17) 1930.

Eusterman [15] recently reviewed a series of 93 cases of gastric syphilis. Regarding the association of hemorrhage in this connection, he said:

> Although I realize that there have been authentic reports of cases of severe gastro-enteric hemorrhage, including those of acute perforation, there are only five trustworthy instances of bleeding in this entire series. In two of these there was definite evidence of associated hepar lobatum, and in the others, undoubtedly, syphilitic changes in the liver had taken place, as is the rule. This lesion might have played a considerable part in the production of the hemorrhage, as Chiari has pointed out. Obliteration of the arteries and veins in actual syphilitic gastric lesions and the tendency to fibrosis and atrophy, or to degeneration of the parenchyma, are undoubtedly large factors in preventing gross or occult bleeding. Hemorrhage is more characteristic of ulcer and carcinoma. In only fourteen cases was anemia present. This was of the secondary type and usually was not marked.

There is, of course, the possibility that, in the case reported here there was sufficient injury to the liver so that increased portal block with varices must be borne in mind as a possible explanation for the hemorrhage.

SUMMARY

Intrinsic gastroduodenal lesions, that is, peptic ulcer, gastric carcinoma, inflammatory processes and benign tumors, accounted for approximately 90 per cent of 668 cases of hematemesis.

The most common single lesion responsible for hematemesis is peptic ulcer, including duodenal, benign gastric and anastomotic ulcers, which comprised 85 per cent of the cases of hematemesis due to intrinsic gastroduodenal lesions.

Carcinoma of the stomach was the etiologic factor in only 12.6 per cent of the cases of hematemesis. Hematemesis usually appears like coffee-grounds in gastric carcinoma, and the bleeding is rarely profuse. Massive hematemesis occurs in only 1 per cent of cases of gastric carcinoma.

Localized or diffuse areas of inflammatory reaction of nonspecific character in the stomach, duodenum or surrounding a gastro-enteric stoma may lead not only to symptoms suggestive of peptic ulceration but to hemorrhage, and yet such areas may not produce sufficient abnormality in the contours or functions of these organs to be recognizable roentgenologically. Such an inflammatory area may be one of the underlying causes of bleeding previously described as gastrostaxis by Hale White and others.

Benign tumors of the stomach and duodenum and gastric tuberculosis and syphilis are rare causes of hematemesis, and their diagnosis usually depends on roentgenologic or surgical observation.

The mechanism of bleeding of the various intrinsic gastroduodenal lesions causing hematemesis has been considered.

---

15. Eusterman, G. B.: Gastric Syphilis, J. A. M. A. **96**:173 (Jan. 17) 1931.

SICKLE CELL ANEMIA

REPORT OF A CASE

G. M. BRANDAU, M.D.
Associate Physician, Jefferson Davis Hospital
HOUSTON, TEXAS

Sickle cell anemia, since its description by Herrick [1] in 1910, has become established as a well defined disease entity. The clinical picture is so distinctive that a tentative diagnosis of the condition can be made from the history and physical signs before the examination of the blood is completed. While the disease is relatively frequent in some sections of the country, perhaps on account of its familial tendency and the drawing power of the more celebrated clinics, nevertheless, it is rare in this vicinity, the only other case in Houston brought to my attention, besides the one to be reported, having been published by Milliken [2] in 1928. Sydenstricker [3] reported a ratio of 1 case to every 400 Negro patients seen in his clinic, but the incidence in this locality is almost certainly not so high, as I and my associates have been constantly on the lookout for this type of anemia with only the modicum of success indicated. The sickle cell trait, however, maintains practically the same percentage here as elsewhere, showing but little variation in its national distribution, if one is to judge by the few reports that have been published. In 150 Negro workers studied by me [4] the incidence was found to be 6.67 per cent; this number was subsequently increased to over 200, with an incidence of 6.5 per cent. Some confusion has arisen through want of a uniform classification of the subject. Sydenstricker objected to the classification proposed by Huck (symptomless, mild and severe) on the grounds that the so-called symptomless cases are in reality not symptomless, and suggested that the cases be designated as latent or active. However, as pointed out by Hahn,[5] it would seem inconsistent with present usage to say that a person who is not now anemic, and has never evidenced any tendency to be anemic, has a case of latent anemia. He therefore used the term sickle cell trait in referring to the condition in

1. Herrick, J. B.: Peculiar Elongated and Sickle-Shaped Red Blood Corpuscles in a Case of Severe Anemia, Arch. Int. Med. **6**:517 (Nov.) 1910.
2. Milliken, Gibbs: M. Rec. & Ann. **22**:49 (March) 1928.
3. Sydenstricker, V. P.: Sickle Cell Anemia, South. M. J. **17**:177 (March) 1924.
4. Brandau, G. M.: Am. J. M. Sc. **180**:813 (Dec.) 1930.
5. Hahn, E. V.: Am. J. M. Sc. **175**:206 (Feb.) 1928.

which sickling occurs in moist blood films, but in which there is no reduction in the erythrocyte count and no history to indicate previous hemolytic attacks. The following classification, a composite of those already outlined, is offered, and has the advantage that all cases in which sickling takes place can be definitely assigned to some one division or subdivision according to the present knowledge of the subject.

I. Sickle cell trait; healthy persons with sickling in vitro
II. Sickle cell anemia
    1. Latent; healthy persons who are subject to recurring periods of active sickle cell anemia
    2. Active
        (*a*) Mild; slightly or moderately anemic patients with sickling in vitro or both in vivo and in vitro
        (*b*) Severe; patients with severer grades of anemia with sickling in vivo and in vitro

The word healthy is used in respect to sickle cell anemia and not to exclude other unrelated conditions.

The literature on sickle cell anemia has been rather sparse, as is shown by reference to the *Quarterly Cumulative Index Medicus,* and, aside from the case reports of Huck [6] and of Sydenstricker,[7] the number of clinical studies has been few. On account of the comparative rarity of the disease and the points of especial interest presented by my case, it seems justifiable to describe the findings in some detail.

REPORT OF A CASE

History.—I. H., a Negro, aged 22, tall, poorly developed and poorly nourished, was admitted to the Jefferson Davis Hospital on Oct. 28, 1931 (in the service of Dr. F. H. Kilgore), with the diagnosis of acute endocarditis and tertiary syphilis. On admission his complaints were: (1) shortness of breath, (2) swelling of the feet and ankles, (3) general weakness, (4) increased frequency of urination, (5) dull pain throughout the entire chest and (6) ulcers on the right leg.

The present illness began five months before admission when the patient was troubled with swelling and aching of the wrist and ankle joints. He called this trouble rheumatism. Pain in these joints continued and grew worse; his knees became sore and painful but did not swell, and three weeks before admission he was confined to bed with shortness of breath, edema of the feet and ankles and pain in the chest. The sores on his leg, however, had been present for eighteen months or longer. He had not felt well since the first of the year and had been having fever all this time. The urine had been highly colored, and at times there was burning on urination. Sometimes his symptoms were intensified when the urine was highly colored.

The patient's mother had told him that he was a normal, healthy baby until he was 9 months of age, but that since then he had never been in robust health. His

6. Huck, J. A.: Bull. Johns Hopkins Hosp. **34**:335 (Oct.) 1923.

7. Sydenstricker, V. P.: Sickle Cell Anemia, J. A. M. A. **83**:12 (July 5) 1924; (footnote 3).

birth and early infancy were not marked by any unusual incident, but he was unable to state whether or not the onset of symptoms antedated the period of weaning. He had had measles and whooping cough when a child, but had escaped the other infectious fevers of childhood. When he was a small boy he suffered from rheumatism, and he stated that he had been jaundiced all his life. When about 13 years of age he began to have ulcers on his legs, and these continued for several years, finally healing, only to reappear at the age of 20. The year before examination he had an attack of severe pain in the abdomen which lasted about a week, and which was not accompanied by diarrhea or vomiting. He was not attended by a physician at that time, so no diagnosis was made. He said that he had never had a venereal infection; he had never been married, but he considered his libido and sexual power to be normal when he was not acutely ill.

His father was living and well. His mother died of a miscarriage when he was 5 years of age. One brother and three sisters were living and well. Several siblings had died in infancy. No history of familial disease could be elicited; especially had there been no rheumatism, jaundice or disease similar to the patient's illness. All other members of the family as far as the patient knew or could remember had been strong, robust people.

Physical Examination.—The patient was a somewhat emaciated adult Negro, lying quietly in bed, but evidencing some distress from dyspnea and exhibiting the harassed facies of the person with cardiac decompensation. His extremities were long in proportion to his body. The skin was dry and intensely black. The palms and soles and mucous membranes showed a distinct pallor. Although tall, he was of slight build and his muscles were poorly developed. There was a generalized lymphadenopathy, the cervical, axillary, epitrochlear and inguinal nodes being palpable on both sides. The patient appeared somewhat younger than his stated age of 22 years, resembling more a youth of 17 or 18. The blood pressure was 130 systolic and 45 diastolic.

The scalp was covered by a thick growth of short, kinky hair, although the beard was scanty. The eyes were prominent, showing slight exophthalmos, but the most remarkable ophthalmic finding was a peculiar greenish hue of the sclerae. The palpebral conjunctivae exhibited marked pallor. The external ocular movements were normal, and von Graefe's sign was negative. The teeth were in fairly good condition but poorly kept. The right tonsil was large, the left of normal size; the mucous membranes were pale.

Pulsations synchronous with the heart beats were observed on both sides of the neck. The lymph nodes were enlarged enough to be palpable, but there was no evidence of enlargement of the thyroid.

There was moderate scoliosis, with resultant distortion of the chest. There was also a slight, well rounded kyphosis. A diffuse impulse could be seen and felt over the cardiac area with each heart beat. Dulness extended 2 cm. beyond the nipple line on the left and 2 cm. beyond the external border of the sternum on the right. On auscultation, a to-and-fro murmur was heard over the whole precordium. The pulmonary second sound was accentuated and distinctly louder than the aortic second sound. The heart beat was regular and rapid. The area of hepatic dulness was increased upward. The lungs, as well as could be judged with the thoracic deformity, expanded about equally. Tactile and vocal fremitus were increased over the left lower lobe, where also there was dulness on percussion and increased vocal resonance. Moist râles could be heard in both bases, with bronchial and bronchovesicular breathing over the left lower lobe.

The abdomen was scaphoid. The spleen was not palpable. The lower border of the liver could be felt a fingerbreadth below the costal margin in the midclavicular line, and the liver was distinctly tender.

The arms and legs were emaciated. Both legs had saber shins. On the left leg was the scar of a healed ulcer. On the anterior surface of the right leg there were two ulcers, one about 6 cm. in diameter, the other about 3 cm. These ulcers were circular and shallow and had clean bases with only a small amount of discharge. The skin over the lower half of the legs and over the feet and ankles was hard and wrinkled. There was a moderate amount of edema of the ankles.

The genito-urinary organs appeared normal, but the pubic crines had a feminine distribution.

The reflexes were normal. Mentally, the patient was bright, his mind being alert and active.

Laboratory Examination.—Oct. 28, 1931: Urinalysis on the day of admission showed a straw-colored urine with a specific gravity of 1.004, an alkaline reaction and a trace of albumin. Microscopically, amorphous urates and occasional epithelial cells were seen. Examination of the blood on the same day revealed: hemoglobin, 40 (Sahli); color index, 1; erythrocytes, 2,000,000; leukocytes, 14,400; poikilocytes, many; anisocytosis, marked; coagulation time, three minutes, and bleeding time, two minutes and ten seconds. The polymorphonuclear neutrophils were 72 per cent; small lymphocytes, 28 per cent, and normoblasts, 2 per hundred leukocytes. There was marked achromia.

Oct. 29, 1931: The Wassermann and the Kahn tests were negative on the following day.

Oct. 30, 1031: The blood poikilocytes were many, and anisocytosis was marked; there were 3 normoblasts per hundred leukocytes.

Oct. 31, 1931: Examination of the spinal fluid showed it to be clear and colorless, with globulin not increased, cell count of 3, negative Wassermann reaction, and a mastic reaction of 0000000000.

Nov. 4, 1931: There was no reaction to the van den Bergh test. Schlesinger's test for urobilin gave negative results, and the reaction for bile was negative; however, on November 6, both reactions were positive. At this time, the feces were negative for parasites and ova.

Nov. 10, 1931: The urine was negative for bile on this day. Ehrlich's test for urobilinogen gave a cherry red reaction. The van den Bergh indirect reaction of the blood serum for bilirubin returned 0.487 mg. per hundred cubic centimeters of serum.

Nov. 13, 1931: Examination of the blood showed: creatinine, 0.91 mg.; sugar, 102.4 mg.; urea nitrogen, 23.1 mg., and nonprotein nitrogen, 45.7 mg. The hemoglobin was 38 (Sahli); color index, 0.95; erythrocytes, 2,000,000; leukocytes, 16,800; poikilocytes, many; anisocytosis, marked; platelet count, 480,000; polymorphonuclear neutrophils, 84 per cent; small lymphocytes, 12 per cent; eosinophils, 1 per cent; basophils, 1 per cent; mononuclear neutrophils, 2 per cent and normoblasts, 4 per hundred leukocytes; achromia was marked.

Nov. 14, 1931: On the following day the gastric contents showed free hydrochloric acid after an Ewald test meal.

Nov. 15, 1931: The phenolsulphonphthalein test of kidney function showed: first hour, 25 per cent; second hour, 20 per cent, with a total of 45 per cent. The fragility test revealed erythrocytes. Hemolysis of the patient's cells began in a tube containing 0.3 per cent of salt solution and was not complete in the last dilution of 0.28 per cent. Hemolysis of normal cells began in a tube containing 0.42 per cent of salt solution and was complete in one containing 0.34 per cent.

Dec. 2, 1931: Examination of the blood showed: hemoglobin, 40 (Sahli); erythrocytes, 2,100,000; leukocytes, 6,600; poikilocytes, many, and anisocytosis, marked; polymorphonuclear neutrophils, 59 per cent; small lymphocytes, 36 per cent; large lymphocytes, 4 per cent; basophils, 1 per cent, and marked achromia.

Dec. 4, 1931: The basal metabolic rate on December 4 was + 8.

Dec. 5, 1931: Chemical analysis of the blood by Dr. H. O. Nicholas of Rice Institute showed: calcium, 12.5 mg.; phosphorus, 5.3 mg., and chlorides as sodium chloride, 737 mg. per hundred cubic centimeters of serum.

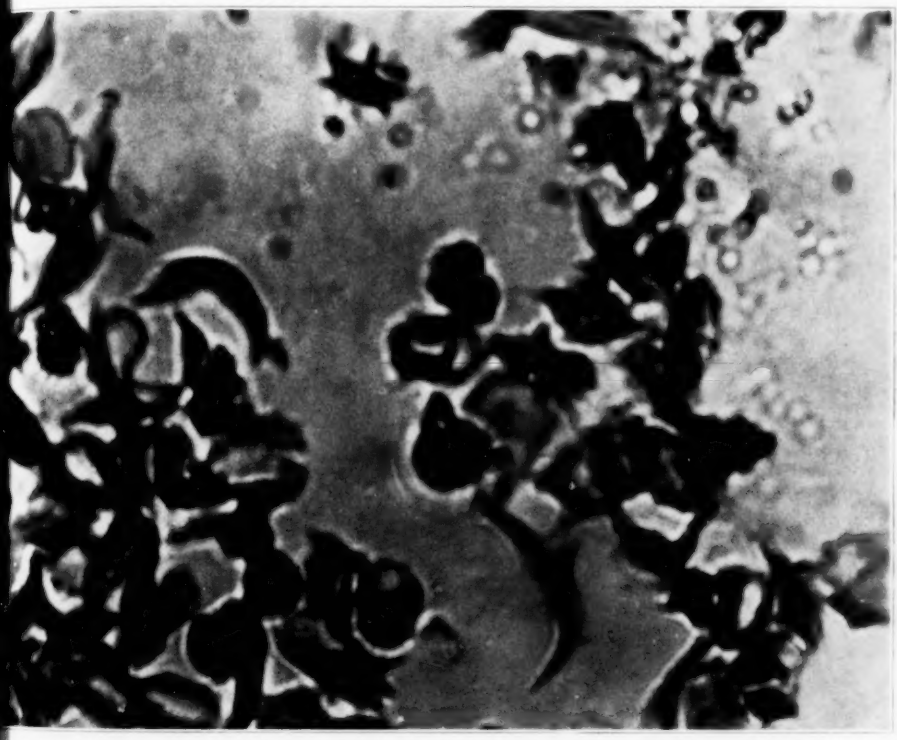


Fig. 1.—Dry stained smear showing sickle cells. Moist films after twenty-four hours exhibited a high percentage of drepanocytic forms.

Dec. 9, 1931: The hemoglobin of the blood was 43 (Sahli); the erythrocytes were 2,550,000; leukocytes, 12,200; polymorphonuclear neutrophils, 74 per cent; small lymphocytes, 26 per cent, and there were many sickle cells.

Dec. 17, 1931: The hemoglobin of the blood had changed to 50 (Sahli) and there were 2,300,000 erythrocytes.

Dec. 21, 1931: A blood smear for reticulocytes showed 6.3 per cent.

Interpretation of the roentgenograms was made by Dr. B. T. Vanzant.

Oct. 29, 1931: That of the chest showed scoliosis and kyphosis, causing considerable deformity of the chest. The heart shadow was enormously enlarged—pyriform—indicating pericarditis with effusion. The lungs showed hypostasis, especially the base of the right lung, probably hypostatic pneumonia.

Oct. 31, 1931: A roentgenogram showed at this time an enormously dilated heart with hypostatic congestion in both lungs.

Fig. 2.—Roentgenogram of the chest showing the enormously dilated heart.

Nov. 19, 1931: A roentgenogram of the skull on November 19 showed a moderate amount of striation in the parietal margin, numerous rounded areas of absorption near the vertex, suggesting pachymeningitis. The mossy, striated appearance between the two tables of the skull, the parietal region, is frequently observed in sickle cell anemia, but is not always present. The same appearance is encountered in numerous other conditions. The rounded areas of resorption are similar to those found in metastatic malignancy. The appearance differs slightly from that of metastasis, the margins being more clearly defined and the distribution corresponding more nearly to the area of the greatest frequency of the pacchionian bodies.

Nov. 23, 1931: A roentgenogram of the legs showed slight bowing of both tibiae without definite thickening of the crest. The medullary portion of the bone showed irregular areas of splotching with a distinct loss of density, the results of anemia.

Treatment and Progress.—On admission, there was a pyrexia of 101 F. During the first two weeks in the hospital, the temperature fluctuated between this point and 99 F., with occasional fractional variation above or below these figures. In the third week it varied from 98.6 to 99.6 F., and toward the end of the fourth week remained normal, continuing so, except for a two day flare-up reaching 100.6 F., throughout the remainder of hospitalization. The pulse rate varied

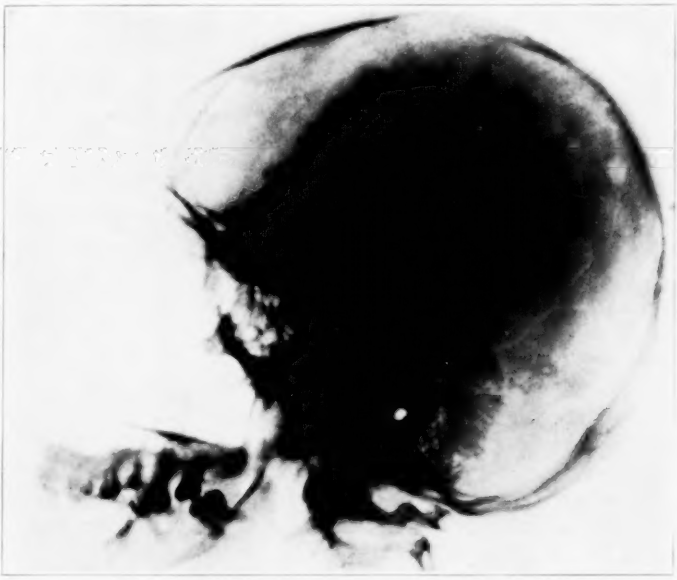


Fig. 3.—Rounded areas of resorption can be seen in the occipitoparietal region. The striations between the tables of the skull which are present in the original roentgenogram do not show plainly in the reproduction.

between 100 and 128 until the third week, when it remained at 100, after which it fluctuated between 80 and 100 until the patient left the hospital. On entering the ward he was given a diet low in protein with the intake of fluid limited to 1,000 cc. per day. Digitalis and ammonium nitrate therapy was instituted, and morphia was given to permit sufficient rest and sleep and to allay the pain. Fowlers' position was used on account of the difficulty in breathing. The edema subsided rapidly, and dyspnea and pain diminished, so that after the first week the patient rested well though still complaining of some shortness of breath. The area of consolidation in the left lung cleared up as compensation of the heart was established, and the moist râles disappeared. Clinically, this was not pneumonia, but was produced by compression of lung tissue by the enormously dilated heart in the

same manner that like signs are produced in the lung above a pleural effusion. The hypostasis was no doubt a result of passive congestion; while it was present, the patient was subjected to a harassing cough.

Although enlargement of the liver is an integral part of the symptom complex of sickle cell anemia, passive congestion due to heart failure was sufficient cause to

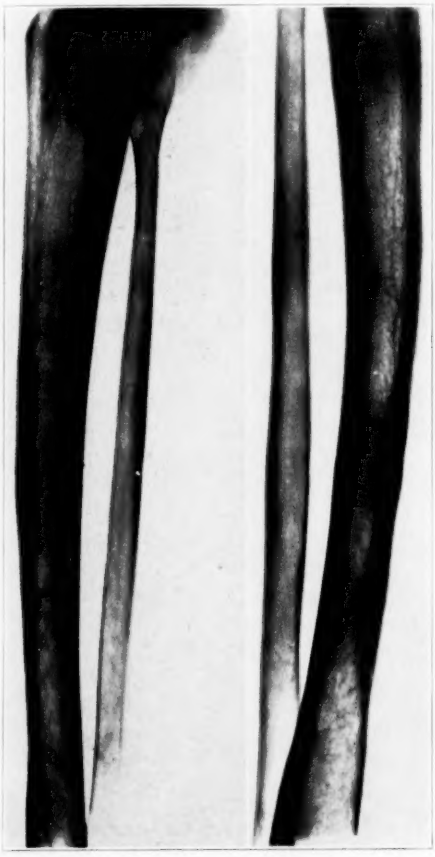


Fig. 4.—Roentgenogram of the bones of the legs showing the decreased density and splotching of the medullary portions. At this angle the bowing of the tibiae is not much in evidence.

account for the hepatic signs in this case, for as compensation was restored, the liver returned to normal size and the tenderness disappeared.

The ulcers on the leg were dressed with ammoniated mercury, U. S. P., and healed fairly promptly. Rest in bed, however, probably had more influence in obtaining this result than did the medicament employed.

Treatment with mercury succinimide and saturated solution of potassium iodide was begun on October 31. One-sixth grain of the mercury preparation was injected into the gluteal muscles every day for six days, and the latter was given in 15 minim (1.93 cc.) doses three times a day. On November 26, fresh spleen was added to the patient's diet, for, as the spleen is atrophic [8] in these advanced cases, it seemed possible that splenic pulp might prove of benefit. A soft diet with spleen was continued without significant improvement in the patient's condition until December 21, when spleen was discontinued and ventriculin with iron was ordered. The patient asked for his discharge on December 23, so that we were not permitted to observe the effect of the latter medication. Splenectomy was considered and deemed inadvisable.

On November 7, the notes on the patient's progress described a pericardial rub. Two days later this was still audible but much softer, and on the following day could not be heard. On November 9, the patient complained of partial inability to use his left arm; the next day he could use it better, and after that he made no further complaint in this regard. From November 28 to December 1, he had diarrhea and fever. Bismuth and camphorated tincture of opium checked the diarrhea, and the fever subsided. On December 7, he complained of burning on urination. On December 9, the blood pressure was 110 systolic and 70 diastolic. The remainder of the patient's stay in the hospital was uneventful; he improved slowly and without any marked change in the erythrocyte count.

COMMENT

In the history it is interesting to note that the age of onset was definitely placed at 9 months. The symptoms that the patient exhibited at that time could not be determined, except that while formerly a healthy vigorous baby he then became sickly and had poor health. As he grew older, the symptoms were typical of his disease. The periods of burning on urination and of highly colored urine did not, as in some cases, always coincide with exacerbations of the illness, and the paroxysm of this nature that he suffered while under observation was not marked by intensification of symptoms or by significant change in the blood count. Abdominal crises were less frequent than in many cases, the history of only one being elicited. Unfortunately, I was not able to examine the blood of the patient's relatives who live in the country some distance away.

The slender build, youthful appearance and general lymphadenopathy are common findings in sickle cell anemia, and the green color of the sclerae, though not always present, is one of the diagnostic signs. It was thought that the bony deformities may possibly have been due to anemia in early life at a time when the osseous structures were normally soft, and that they were made more so by the impoverished state of the blood. The roentgen appearance at the present time tends to support such a hypothesis and to rule out syphilitic osteoperiosteitis.

8. Yater, W. M., and Mollari, Mario: Pathology of Sickle-Cell Anemia: Report of Case with Death During "Abdominal Crisis," J. A. M. A. **96**:1671 (May 16) 1931.

Extinct rickets as a cause of the bony changes seems unlikely, as there is only a forward bowing of the tibiae and neither epiphyseal thickening or a rachitic rosary.

SUMMARY

Sickle cell anemia is a comparatively rare disease; sickle cell trait is more common, and apparently more evenly distributed. A classification of the conditions in which sickling of the red blood cells occurs is offered in the hope that its use will obviate the confusion that has heretofore existed. A case of sickle cell anemia is reported and commented on, and the progress and treatment discussed. The case, while typical, presents some interesting individual features represented in the physical signs and in the roentgen and laboratory data. After the administration of splenic pulp, improvement occurred, but this was not marked enough to be significant. Treatment with mercury and iodides seemed to be of value but not specifically so. Study of the case was interrupted by the patient's departure from the hospital as improved.

Book Reviews

Diseases of the Coronary Arteries. (Myocarditis). By Don C. Sutton, M.S., M.D., Associate Professor of Medicine, Northwestern University; Attending Physician, Cook County Hospital; Chief, Cardiac Follow-up Clinic, Cook County Hospital, Chicago, and Harold Lueth, Ph.D., M.D., Formerly Instructor of Physiology, Northwestern University, Chicago. Cloth. Price, $5. Pp. 164, with charts and illustrations. St. Louis: C. V. Mosby Company, 1932.

Disorders of the coronary circulation have always been matters of great interest to physicians. Since the time of Heberden and Jenner the amount of study and investigative work devoted to this subject has been tremendous. In this book Sutton and Lueth have reviewed this vast amount of material and have covered the field in a way to be of great value to the reader. The mass of statistical data is unfolded so rapidly and the book so abounds with references to the literature that the orderly discussion of the subject is somewhat obscured.

The first chapter is devoted to the symptomatology of heart failure. The authors rather apologetically touch on acute myocarditis, and, indeed, it adds nothing to the book. The term itself is a difficult one to justify, and an attempt to deal with it in two scant pages is foredoomed to failure. The advisability of dealing with rheumatic fever under this heading and in one short paragraph is questionable. The section on chronic myocarditis is dealt with more adequately, because it is definitely set off as the result of arteriosclerosis of the coronary vessels. While this definition may be questioned, it forms a limitation to the field of discussion.

The arrhythmias are well set forth, although it is possible that the authors overemphasize the importance of the significance of ectopic contractions in patients over 40. They also totally disregard the possibility that auricular fibrillation may accompany minor pathologic changes, or may even occur in hearts apparently normal anatomically.

In the section on angina pectoris, the excellent work of the authors on the mechanism of the production of anginal pain is dealt with. This work is an exceedingly valuable contribution to the study of coronary disorders. It is unfortunate, however, that the authors have made no attempt to differentiate angina pectoris from anginal pain and have continued to confuse angina pectoris with coronary thrombosis. In one of the illustrative cases there is described under the head of angina pectoris an attack which lasted from Sunday until Tuesday. A few pages further on the same case is described as a coronary thrombosis, which it proved to be at autopsy. Since Herrick separated from the heterogeneous mass of anginal pains the clinical entity now known as coronary thrombosis, one is able to select from the literature many cases described as angina pectoris that were undoubtedly thrombosis of the coronary artery. It would seem that the present knowledge of angina pectoris with its sudden onset and offset would permit one, with a little courage, to separate it from the remaining anginal pains and allow it to occupy a position of its own.

There is a full discussion of the causes of angina pectoris. The authors seem to doubt the presence of a vasoconstrictor mechanism in the coronaries, and yet inconsistently they later mention the vasoconstriction produced by nicotine, the probable vasoconstrictor action of digitalis and the vasoconstrictor responses elicited by the stimulation of certain nerves

The symptomatology of coronary thrombosis is adequately described. In the section on physical examination of the heart, the physical findings are set down.

and electrocardiographic evidence is discussed. Many illustrations taken from the work of Gross aid in the portrayal of the distribution of the coronary circulation, and the field of pathology is well reviewed. The researches of the senior author add much to the value of this section. A chapter is devoted to the drugs that influence the coronary circulation, and the final chapter deals with the treatment of various types of myocardial failure.

There are numerous inaccuracies that keep the book from being the finished product that it should be. Euphyllin or metaphyllin is incorrectly called a theobromine compound. Under the head of focal infection, the authors discuss acute infection, with some lack of clarity. In many places clarity has been sacrificed for condensation.

In discussing the use of digitalis in auricular fibrillation, marked sinus arrhythmia is cited as evidence of an overdosage of digitalis. A respiratory arrhythmia has been noted in auricular fibrillation, but has been considered as evidence of an optimum dosage. The dose of some of the recommended drugs is given, and theocin and its sodium acetate are recommended in doses of from 10 to 20 grains (1.3 to 1.9 Gm.). Such doses have been known to produce convulsions.

The book contains many excellent illustrations, and its value is further enhanced by the use of numerous illustrative cases. The intrinsic value of the book more than offsets the portion open to possible criticism.

Verhandlungen der Deutschen Gessellschaft für Kreislaufforschung. IV. Tagung, gehalten zu Breslau am 9. und 10. März, 1931. Herausgegeben von Prof. Dr. Bruno Kisch, Köln. Price, 15 marks. Pp. 242, with 53 illustrations. Dresden: Theodore Steinkopff, 1931.

The fourth session of the Deutschen Gesellschaft für Kreislaufforschung was devoted almost exclusively to a consideration of the experimental and clinical studies of digitalis that have been under investigation by German workers during recent years. This group of papers commends itself as a valuable contribution to the knowledge of digitalis. It is more than that; it is a concise presentation of the views now dominant in Germany regarding the indications for the use of digitalis, the proper methods of administration and its relative value as compared with its congeners, especially strophanthin. The latter drug is evidently much more widely used in Germany than in this country, and, one may add, it is better understood. As will happen in the transactions of any society, the papers differ from one another in glory. They are not all of equal value. Even so, this little volume is filled with information, stimulation and, in parts, fascination. The referat of Pribram on "Digitalistherapie" is excellent. It is a comprehensive review of the problems of present-day digitalis therapy, and is filled with suggestive and stimulating observations. Professor Schaffer's discussion of the arguments for and against the use of large doses of digitalis brings out the feeling that prevails in Germany regarding the Eggleston method of digitalis dosage. The point of his argument, which is emphasized by others, is essentially expressed in this sentence, "The central point of every course of treatment with digitalis is and remains individual dosage." His objection to the American method is that it does not meet the variable need for digitalis of the diseased heart. The use of strophanthin is evidently much better established there than in this country, and the indications for this use are more clearly defined, although the paper of Filip on the indications for the use of strophanthin is not altogether convincing. One of the longer articles is on the subject of "The Theory of the Digitalis Effect," by Otto Krayer. There are a few articles concerned with digitalis and electrocardiography, several on various other phases of digitalis investigation and others on subjects not related to digitalis. One of the most interesting of the latter is a long paper by Gruber on "Vascular Disease and Gangrene." This volume may be commended to all who are interested in the advances in the field of cardiac disease. It is exceptionally interesting and valuable.

Traité de physiologie, normale et pathologique. Publié sous la direction de G. H. Roger, Professeur honoraire de physiologie à la Faculté de médecine de Paris, et Léon Binet, Professeur de physiologie à la Faculté de médecine de Paris. Tome II. Alimentation et digestion. Par E. Bardier, G. Battez, H. Bierry, Léon Binet, P. Garnot, P. Combemale, C. Delezenne, A. Desgrez, R. Gayet, R. Glenard, L. Hallion, M. Lisbonne, A. Pi Suner, M. Vagliano et E. Vollmann. Price, 100 francs. Pp. 566. Paris: Masson & Cie, 1931.

The authors listed contributed articles to this volume on the following subjects: foods and rations, vitamins, hunger, thirst, the salivary glands, the stomach (chiefly its secretory function), the intestine (secretion and digestion), the external secretion of the pancreas, intestinal absorption, bacteria and bacterial action in the digestive tube, mastication and deglutition and the movements of the stomach and intestine. A few of these discussions are excellent, especially those dealing with hunger, thirst, the digestive ferments and the effect of bacterial action, but the remainder are mediocre. The presentation consists chiefly of the ordinary facts of physiology established prior to five years ago, with a striking lack of reference to work done since then. The findings of American investigators have received relatively little attention.

The type is readable, and the binding is excellent; illustrations are scarce. It is pleasing to note the brief historical reviews and the numerous references to the early workers in the various fields. These compensate in a measure for the omission of more recent material. The volume is of considerable value as a reference work.

A Textbook of Laboratory Diagnosis with Clinical Applications for Practitioners and Students. By Edwin E. Osgood, M.A., M.D., Assistant Professor of Medicine and Biochemistry, University of Oregon Medical School, and Howard D. Haskins, M.D., Professor of Biochemistry, University of Oregon Medical School. Cloth. Price, $5. Pp. 475. Philadelphia: P. Blakiston's Son & Co., 1931.

This splendid textbook is an outgrowth of an outline which has been used for years in teaching laboratory diagnosis at the University of Oregon Medical School. The subject matter has been divided into two natural divisions: Part 1 is a consideration of that body of knowledge which the practitioner must have available at the bedside of the patient. Part 2 is a consideration of the knowledge necessary for use in the laboratory.

The subject matter has been correlated by systems for practical purposes and to stimulate interest and to permit the person interested either in technic or in interpretation to study as a connected whole the phase of the subject that is of greater interest at the moment.

At the beginning of each chapter in part 1, there is a brief résumé of the essential anatomy, physiology, biochemistry and pathology of the system under discussion. Source references are given in the footnotes throughout the book. The illustrations, many of which are in color, are excellent, and there are numerous tables and charts. In addition to the standard type of index, there is an index by diseases which enhances the value of the work as a handy reference volume.

This textbook is so thorough, clear and practical that it should rapidly become one of the "pets" in every student's library; also, the reviewer has not found a more suitable work for rapid review or ready reference for the busy practitioner.

Guide to Radiologic Diagnosis in Heart Disease. By Geza Nemet, M.D., with the aid of the Committee on Research of the Heart Committee. Paper. Price, 35 cents. Pp. 33, with 31 illustrations. New York: New York Tuberculosis and Health Association, 1931.

Criteria for the Interpretation of Electrocardiograms. By Arthur C. DeGraff, M.D., with the aid of the Committee on Research of the Heart Committee. Paper. Price, 35 cents. Pp. 19, with 37 illustrations. New York: New York Tuberculosis and Health Association, 1931.

These two pamphlets constitute a plea for standardization in cardiac diagnosis. The first pamphlet describes the technic of radiologic examination, what may be learned from radiologic examination and, equally important, what may not be determined. It is of equal value to the radiologist and the clinician. Many diagrams depicting the changes that accompany various disorders of the heart add greatly to the value of the work.

The second pamphlet should be entitled "Criteria for the Description of Electrocardiograms." Correctly enough, it does not consider interpretation in the sense that the fundamental cause of the electrocardiogram is discussed. Fifty key numbers are given which may be used to describe the various types of electrocardiographic curves. These cover all of the usual, and many of the unusual, graphs. Illustrative electrocardiograms are included.

The authors express the hope that both volumes may lead to more uniformity in the terms of cardiac diagnosis. Some such medical *esperanto* is surely needed, and these pamphlets constitute a step in the right direction.

Pain in the Pleura, Pericardium and Peritoneum: A Clinical Study. By Joseph A. Capps, M.D., with the collaboration of George H. Coleman, M.D. With an Introduction by Anton J. Carlson, M.D., Ph.D. Macmillan Medical Monograph Series. Price, $3. Pp. 99. New York: The Macmillan Company, 1932.

This monograph consists of a description of concise experiments in the production of pain in the pleura, pericardium and peritoneum with accurate localization of direct and referred pain. The localization is charted and described. In addition, there are clinical reports with case examples of pain, the typical reference of which is explained by the authors' experimental results.

The experimental work is objective, and the experiments themselves are clear-cut and conclusive. The book is valuable for its physiologic and anatomic explanation of referred pain. Its clinical application is one of almost daily experience; the contents deserve the widest dissemination among students and practitioners.

THE ARCHIVES OF INTERNAL MEDICINE is published by the American Medical Association for the advancement of scientific medicine in the United States and as a medium of publication for research and observation in the domain of internal medicine.

Manuscripts for publication, books for review, and correspondence relating to the editorial management should be sent to the editor-in-chief, Dr. N. C. Gilbert, 535 North Dearborn Street, Chicago. Communications regarding subscriptions, reprints, etc., should be addressed, ARCHIVES OF INTERNAL MEDICINE, American Medical Association, 535 North Dearborn Street, Chicago.

Articles are accepted for publication on condition that they are contributed solely to the ARCHIVES OF INTERNAL MEDICINE. Manuscripts must be typewritten, preferably double spaced, and the original copy should be submitted. Zinc etchings and halftones of illustrations will be supplied by the Association when the original illustrations warrant.

Footnotes and bibliographies should conform to the style of the Quarterly Cumulative Index Medicus, published by the American Medical Association. This requires, in order given: name of author, title of article, name of periodical, with volume, page, month—day of month if weekly—and year. A complete list of abbreviations for standard periodicals, together with a full discussion of the style of the A. M. A. publications, appears in The Art and Practice of Medical Writing, a comprehensive book on the preparation of medical manuscripts, published by the American Medical Association. Price, $1.50.

Matter appearing in the ARCHIVES OF INTERNAL MEDICINE is covered by copyright, but, as a rule, no objection will be made to its reproduction in reputable medical journals, if proper credit is given. However, the reproduction for commercial purposes of articles appearing in the ARCHIVES OF INTERNAL MEDICINE, or in any of the other publications issued by the Association, will not be permitted.

Authors will receive one hundred reprints free; additional reprints may be obtained at cost.

The ARCHIVES OF INTERNAL MEDICINE is published monthly. Annual subscription price (two volumes): Domestic, $5.00; Canadian, $5.40; foreign, $6.00, including postage. Single copies, 75 cents, postpaid.

Checks, etc., should be made payable to the AMERICAN MEDICAL ASSOCIATION.

OTHER PERIODICAL PUBLICATIONS
of the American Medical Association

THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION—Weekly. Covers all the medical sciences and matters of general medical interest. Illustrated. Annual subscription price (two volumes): Domestic, $7.00; Canadian, $8.50; foreign, $11.00. Single copies, 25 cents.

AMERICAN JOURNAL OF DISEASES OF CHILDREN—Monthly. Presents pediatrics as a medical science and as a social problem. It includes carefully prepared collective abstracts based on recent pediatric literature, abstracts from foreign and domestic literature, book reviews, society transactions, etc. Illustrated. Annual subscription price (two volumes): Domestic, $8.00; Canadian, $8.40; foreign, $9.50. Single copies, 85 cents.

ARCHIVES OF NEUROLOGY AND PSYCHIATRY—Monthly. A medium for the presentation of original articles on nervous and mental diseases, with abstracts from foreign and domestic literature, book reviews, society transactions, etc. Illustrated. Annual subscription price (two volumes): Domestic, $8.00; Canadian, $8.40; foreign, $9.50. Single copies, 85 cents.

ARCHIVES OF DERMATOLOGY AND SYPHILOLOGY—Monthly. Devoted to advancing the knowledge of and progress in cutaneous diseases and syphilis. Publishes original contributions and full abstracts of the literature on these two subjects, transactions of the important dermatological societies, book reviews, etc. Illustrated. Annual subscription price (two volumes): Domestic, $8.00; Canadian, $8.40; foreign, $9.00. Single copies, 85 cents.

ARCHIVES OF SURGERY—Monthly. Devoted largely to the investigative and clinical phases of surgery, with monthly reviews on orthopedic and urologic surgery. Well illustrated. Annual subscription price (two volumes): Domestic, $8.00; Canadian, $8.40; foreign, $9.00. Single copies, 85 cents.

ARCHIVES OF OTOLARYNGOLOGY—Monthly. A medium for the presentation of original articles on diseases of the ear, nose and throat, with abstracts from foreign and domestic literature, book reviews, the transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): Domestic, $6.00; Canadian, $6.40; foreign, $7.00. Single copies, 75 cents.

ARCHIVES OF PATHOLOGY—Monthly. A periodical devoted to the publication of original contributions in the field of pathology, with abstracts from foreign and domestic literature, book reviews, the transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): Domestic, $6.00; Canadian, $6.40; foreign, $7.00. Single copies, 75 cents.

ARCHIVES OF OPHTHALMOLOGY—Monthly. Includes original articles on diseases of the eye, abstracts from foreign and domestic literature, book reviews, transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): Domestic, $8.00; Canadian, $8.40; foreign, $9.00. Single copies, 85 cents.

QUARTERLY CUMULATIVE INDEX MEDICUS—Quarterly. A complete subject and author index to the worth-while current medical literature of the world. Issued four times a year. Second and fourth volumes bound for permanent reference. Subscription price, calendar year: Domestic, $12.00; Canadian, $12.50; foreign, $13.50.

AMERICAN MEDICAL ASSOCIATION
535 North Dearborn St. **CHICAGO**

CONTENTS

PAGE

Insulin Dosage and Blood Sugar Changes. Ernest L. Scott, Ph.D., and Louis B. Dotti, M.A., New York 511

Pernicious Anemia: Treatment with Equine Liver Extract Injectable Either Subcutaneously or Intravenously. Arthur E. Meyer, Ph.D.; Oscar Richter, M.D., and Andrew C. Ivy, M.D., Chicago 538

Urea Clearance Test as an Index of Renal Function. Maurice Bruger, M.D., and Herman O. Mosenthal, M.D., New York:

III. Studies of Patients with Bright's Disease 544

IV. The Urea Clearance Test in Relation to Other Tests and Measures of Renal Function 556

Sedimentation Test as a Routine Laboratory Procedure: Observations on Eleven Hundred Persons. Herbert J. Schattenberg, M.D., New Orleans 569

Methemoglobinemia. William J. Dieckmann, M.D., Chicago 574

A Method for the Simultaneous Enumeration of Blood Platelets and Reticulocytes, with Consideration of the Normal Blood Platelet Count in Men and in Women. William Dameshek, M.D., Boston 579

Kidney Weight, Body Size and Renal Function. Eaton M. MacKay, M.D., La Jolla, Calif. 590

Nontropical Sprue with Duodenal Involvement and Tetany. Robert B. Radl, M.D., and Madeleine Fallon, M.D., Minneapolis 595

Experimental Studies in Gastric Physiology: Evaluation of the Rôle of Duodenal Regurgitation in the Control of Gastric Acidity in Man (Boldyreff Theory). Harry Shay, M.D.; Albert B. Katz, M.D., and Eugene M. Schloss, M.D., Philadelphia 605

Intrinsic Gastroduodenal Lesions as Causative Factors of Hematemesis. Andrew B. Rivers, M.D., and Dwight L. Wilbur, M.D., Rochester, Minn. 621

Sickle Cell Anemia: Report of a Case. G. M. Brandau, M.D., Houston, Texas 635

Book Reviews 645